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ODYSSEY

Is It Possible That Molecular Interactions of the Trophoblast-Specific Beta-Glycoprotein Could Determine Its Function?

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Hypothesis: Currently the Trophoblast-Specific beta-Glycoprotein (TSG) is used in clinical practice as Indicator of Pregnancy and marker of trophoblastic disease, including Choriocarcinoma. In the early 70's of last century a group of scientists led by Tatarinov identified the role of TSG in the diagnosis of pregnancy, and it was detected in the blood of patient horiocarcinoma. This protein was studied by several research groups, each of which gave him the original name, so in the scientific literature there are many of its symbols (TBG, SP1, PAPP-B, PSBG1, PSG).

Objectives: TSG is synthesized in syncytiotrophoblast, so we can assume that it is specific tissue marker. In the study of the physiological role of this protein was revealed firstly its immunosuppressive function that logically explains the increasement of TSG levels during all pregnancy. Then, there is evidence of its possible association with steroid hormones. We noticed that serum blood TSG can change its electrophoretic mobility and to be identified in the zone of IgG. The fact that different mobility TSG may be due to its interaction with IgG, which can be explained by the fact that TBG carries immunosuppressive function. Based on this, the goal of our work was to study interactions TSG and IgG due to identical clusters of hydrophobic radicals on the surface of their structures.

Methods: We used the amino acid sequence database Uniprot (http://www.uniprot.org/), server I-Phyre (http://www.sbg.bio.ic.ac.uk/phype2/html/page.cgi?id=index), a database of protein structures PDB (http://www.rcsb.org/pdb/home.do), programs Swiss PDB Viewer (http://spdbv.vital-it.ch/) and Yasara (http://www.yasara.org/).

Result: The TSG has highly conserved portions and recess-pocket of protein molecule, wherein may be is an active center. Comparative analysis of the amino acid sequences showed that IgG has from 476 amino acids 230 nonpolar (48%). In TSG from 419 amino acids 196 of nonpolar (47%). Both protein identified clusters. The IgG protein contains 51 clusters (from 2 to 10 amino acids). Structure of TSG has 42 clusters (from 2 to 6 amino acids). Models of interaction between these proteins are constructed.

Conclusions: Results of the study in silico showed that the TSG and IgG are identical on the composition of clusters, which radicals form intermolecular bonds.

Funding Source: Research in the laboratory of Astrakhan State Medical University, Online Web Servers and Bioinformatics Software.

Anticancer Effects Elicited by Combination of Rubus Extract with Phthalocyanine Photosensitiser On MCF-7 Cells

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Hypothesis: Cancer has attracted great attention owing to the serious threat to human life and to the increased growth of various types of malignant tumors. Breast cancer remains the most common cause of cancer-related death in women. Photodynamic therapy (PDT) is a rapidly-developing approach for the treatment of various diseases. The preferential absorption of photosensitizer (PS) alters certain cellular mechanisms, provides a selective cellular devastation and eventually eradicates cancer cells.

Objectives: The combination of PDT with other therapies is a novel approach for cancer treatment and aims to enhance the efficacy of any treatment. The basis of combination therapy is to administer a small amount of sensitizers coupled with plant extracts and still produces photochemical reaction, the activated sensitizers and phytochemicals causing cytotoxicity. Combination therapies may decrease the sensitizer and phytochemicals dosage thereby reducing side effects. The present study was undertaken to evaluate the photosensitizing ability of Rubus fairholmianus root acetone extract (RFRA) in PDT experiments in vitro.

Methods: The RFRA was conjugated with phthalocyanine photosensitizer to augment the therapeutic effects on MCF-7 cells. A relatively low concentration of PS and extract have been used for the conjugation since it induces apoptosis at very low concentrations. The PDT experiments were carried out using diode laser of wavelength 680 nm with 5, 10 and 15 J/cm2 fluencies. The cells were treated with RFRA and conjugated RFRA-PS for 24 h and analyzed for the changes in morphology, viability, proliferation, cytotoxicity and apoptosis inducing properties.

Result: The PDT groups showed significant features of apoptosis by variation in morphology with loss of cell number, formation of apoptosis bodies and detachment of cells. The viability (51.25% for the high dose PDT) and Adenosine 5′-triphosphate (ATP) proliferation rates of treated cells reduced considerably with increased cytotoxicity by lactate dehydrogenase (LDH) release. The Annexin V/PI flow cytometric analysis strongly supports the results of caspase 3/7 activities by increasing the percentage of apoptotic cells population.

Conclusions: In this study, the PDT treatment was enhanced by conjugation of phthalocyanine with Rubus extract. These results indicate the phototoxic effects of Rubus and PS through the caspase dependent apoptosis and it can be concluded that Rubus may be a potent anticancer plant with phototoxic effects on breast cancer cells. The in vitro experiments showed phototoxic activity of Rubus similar to that of other commonly used photosensitizers. This is of utmost interest due to the use of underexplored plant for the treatment of breast cancer. Owing to its cytotoxicity and apoptotic inducing abilities, this plant may be of clinical interest for PDT of cancer.

Funding Source: University of Johannesburg, Laser Research Centre

Mutant p53 as a New Therapeutic Target for Breast Cancer Treatment

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Hypothesis: TP53 (p53) is the single most frequently mutated gene in human cancer. Overall, p53 is believed to be mutated in approximately in 50% of all human cancers. However, in some of the most difficult to treat cancers such as high grade serous carcinomas, triple-negative breast cancers and non-small cell lung cancer, the gene is mutated in approximately 80% of samples. Clearly, therefore, mutant p53 protein an important candidate target against which to new anti-cancers treatments could be developed.

Objectives: To investigate targeting mutant p53 with APR-246 as a new approach for breast cancer treatment.

Methods: Cell viability was determined using the MTT assay. p53 protein levels were determined using Western blot and ELISA while both p63 and p73 levels were measured by Western blotting. Apoptosis was measured by flow cytometry.

Result: Using a panel of 23 breast cancer cell lines, treatment with APR-246 resulted in significantly lower IC50 values for cell growth inhibition in p53 mutant compared to p53 wild-type cells (p=0.014). Furthermore, a significant inverse correlation was found between IC50 values and p53 protein levels (p=0.0001, r=-0.76). In addition to inhibiting cell proliferation, APR-246 was found to promote apoptosis and inhibit cell migration in a p53 mutant-dependent manner. In an attempt to enhance growth inhibition, APR-246 was combined with different cytotoxic agents. Highly syngeristic growth inhibition was found when APR-246 was combined with eribulin in the 6 different cell lines investigated. Both docetaxel and carboplatin showed an additive effect in combination with APR-246. However, this additive effect was cell line-dependent, i.e., docetaxel plus APR-246 was additive in MDA-MB-453 cells (CI=0.92) cells but not in MDA-MB-468 cells (CI=1.7), while carboplatin plus APR-246 was additive in MDA

Conclusions: Our preclinical work suggests that APR-246 may be a new treatment for patients with breast cancer expressing mutant p53. Based on our finding, either the mutational status of the p53 gene or p53 protein levels may be used to predict response to APR-246. Clinical trials investigating this agent should incorporate either or both of these measurements as potential predictive biomarkers.

Funding Source: Irish Cancer Society

Biomarkers in Colorectal Cancer: Which Ones Are Clinically Useful?

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Biomarkers currently play an essential role in the detection and management of patients with colorectal cancer (CRC). Thus, for screening, either a guaiac-based or immunochemical-based faecal occult blood test may be used. Following a diagnosis of CRC, MSI status and/or detection of mismatch repair proteins may also be used for determining prognosis, especially in patients with stage II disease who are being considered for adjuvant 5-FU-based therapy. For patients diagnosed with stage II or III CRC who may be candidates for further intervention (e.g., liver resection or systemic treatment), in the event of recurrent disease, CEA should be measured at baseline and then every 2 to 3 months for at least 3 years after diagnosis. CEA should also be used in monitoring therapy in patients with advanced CRC receiving chemotherapy. For patients with metastatic CRC who do not exhibit increased serum CEA levels, other markers such as CA 19-9 should be considered for monitoring therapy. Although no biomarkers currently exists for identifying patients likely to benefit from specific chemotherapeutic agents, the mutational status of KRAS and NRAS should be used to predict response to anti-EGFR antibodies (cetuximab and panitumumab). For upfront identification of patients at high risk of suffering from severe therapy-related toxicity, dihydropyrimidine dehydrogenase (DPD) may be measured for predicting toxicity from fluoropyrimidines and uridine diphosphate glucuronyltransferase 1A1*28 (UGT1A1*28) for predicting toxicity from irinotecan. Newly emerging biomarkers for CRC include faecal DNA panels and methylated SEPT9 DNA in screening for CRC and specific multigene signatures (OncotypeDX Colon Cancer Assay, GeneFx Colon, ColoPrint Colon Cancer Recurrence Assay) for predicting outcome in patients with stage II disease.

Reference

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Protocol: Detection of False FIT Negative Subjects by Combinations of Blood-Based Biomarkers

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Hypothesis: Combination of various blood-based biomarkers will identify false negative subjects from the FIT screening program.

Objectives: To establish a combined blood-based biomarker profile that identifies subjects with neoplastic bowel lesions although screened negative in the FIT program.

Methods: Within a two-year period 30,000 subjects screened negative by FIT screening will be identified within 14 days after achieving the result. Subsequently, the subjects will be invited by electronic mail to participate in a study with blood collection and data recording at one of 10 participating hospitals. The blood will be collected, handled, and stored at -80oC under 24/7 electronic surveillance according to a well-validated SOP. Data of every included subject will be included into web-based database and audit will be performed currently during the entire study period. Additional blood collection and data recording will be performed after two and four years, respectively, simultaneously with the subsequent FIT screening test. Available blood samples per collection: 10 ml of serum, 25 ml of EDTA plasma, and 3 ml of buffy-coats. The determinations will include proteomes, genomes, epigenomes, transcriptomes and metabolomes.

Result: All subjects will be followed by using the central personal registration number (cpr.nr.) given to all Danish citizens. Thereby the biomarkers may be compared with presence and/or development of neoplastic diseases. Estimated numbers of subjects with diseases are: 150 with presence of CRC (false negative), 800 with presence of adenomas (false negative), 250 with presence of other malignancies, 450 with interval CRC, 1.200 with interval adenomas, 850 with interval extra-colonic malignancies. In addition, velocity issues will be in focus. Finally, calculations will be performed to identify a frequency of out-patient follow-up of at risk subjects.

Conclusions: The current major study may show valuable results in identification of false FIT negative subjects, subjects at risk of interval neoplastic diseases, velocity issues, and the frequency of out-patient examination of at risk subjects.

Funding Source: Danish and European Private Funds, International Biotech Companies, Danish Government Foundations, and Hvidovre Hospital

Increased Serological Protein Biomarker Levels at Diagnostic Bowel Endoscopy Are Associated with Risk of Being Diagnosed

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Hypothesis: Patients diagnosed with adenomas at bowel endoscopy and increased serological protein biomarker levels have a subsequent increased risk of being diagnosed with malignant diseases.

Objectives: To evaluate whether increased serological protein biomarker levels may aid in identifying patients at increased risk of being diagnosed with subsequent primary malignancies.

Methods: The plasma levels of CEA, CA19-9, TIMP-1 and serum levels of YKL-40 were determined in blood samples collected before diagnostic bowel endoscopy. Ten-year follow-up identified patients, who subsequently developed a primary malignant disease; both intra- and extra- colonic malignant diseases were included. The biomarker levels were also determined in 400 age and gender matched subjects with no known co-morbidity, who underwent diagnostic colonoscopy and were identified with clean colorectum; these levels were used to construct the reference intervals. For this exploration, biomarkers were indicated as elevated when levels were above these reference intervals. The 5-year incidence and death was included as a competing risk.

Result: Primary malignancies were identified in 175 of 923 patients; 20 of the 175 patients developed colorectal cancer and 155 developed non-CRC cancers. Three groups were established: 0: no increased biomarkers; 1: 1 of the 4 biomarkers increased; 2: ≥2 biomarkers increased. The cumulative 5-year incidences were: 0: 6.9%; 1: 11.8%; 2: 17.5% (p=0.0009).

Conclusions: An increased protein biomarker level at bowel endoscopy identifies adenoma patients at increased risk of being diagnosed with subsequent primary malignancies.

Funding Source: N/A

Early Detection of Noeplasia: Combination of 17 Cancer-Associated Blood-Based Protein Biomarkers

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Hypothesis: Serological protein biomarkers may detect colonic and extra-colonic cancers in subjects scheduled for diagnostic colonoscopy.

Objectives: To detect colonic and extra-colonic malignancies using cancer associated serological protein biomarkers.

Methods: Subjects scheduled for diagnostic colonoscopy were included. Criteria for inclusion were age ≥18 years, symptoms of colorectal neoplasia and signed informed consent. Exclusion criteria were previous malignant disease, previous large bowel adenoma, previous large bowel endoscopy, surgery within 3 months and member of a HNPCC family. Blood samples were collected before colonoscopy and EDTA plasma levels of AFP, B2M, CA 125, CA 15-3, CA 19-9, CEA, CyFra 21-1, Ferritin, Galectin-3, HE4, HS-CRP, NSE, Pepsinogen 1, Pepsinogen 2, ProGRP, SCC and TIMP-1 were determined by the Abbott ARCHITECT® i2000 immunoassay platform. Outcomes were as follows: 1) Colorectal cancer (CRC) and high risk adenoma, 2) CRC, 3) CRC and extra-colonic cancer and 4) extra-colonic cancer. All outcomes were considered as binary. Both univariable and multivariable logistic regression analyses with the outcomes as the dependent variables and the biomarkers as explanatory variables have been developed.

Result: A total of 4,698 subjects were included. 512 CRCs were included of which 323 were Colonic cancers (CC) and 189 Rectal cancers (RC). We found 699 adenomas of which 298 were high-risk. A total of 177 extra-colonic cancers were found. The univariable analysis estimated AUCs ranging from 0.50 to 0.77 for the respective outcomes. The multivariable analysis produced reduced models of 4 or 6 best biomarkers plus age and gender. The AUCs for Outcome 1 was 0.75 for both the univariable and multivariable models; for Outcome 2 an AUC of 0.84 for both models; Outcome 3 the AUC was 0.82 and 0.81, respectively and for Outcome 4 the AUCs were 0.84 and 0.86, respectively.

Conclusions: Subsets of the 17 biomarkers can be useful for identification of subjects with a high risk of CRC and extra-colonic cancers.

Funding Source: Abbott provided the analyses

The Combination of uPAR(I) And Type IV Collagen Outperforms CEA as A Prognostic Biomarker in Primary Colorectal Cancer

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Hypothesis: The novel serological biomarkers: the soluble urokinase-type plasminogen activator receptor (suPAR) forms (suPAR (I-III), suPAR(I-III)+(II-III) and uPAR(I)), and type IV collagen have significant and independent prognostic as well as predictive value in primary colorectal cancer (CRC).

Objectives: Carcinoembryonal antigen (CEA) is a well established biomarker in CRC providing significant prognostic information. The present study is based on an analysis of the uPAR and type IV collagen biomarkers association to overall survival (OS) adjusted for CEA as well as clinical baseline characteristics, in particular stage II and III patients. The analysis includes interaction between the biomarkers and adjuvant chemotherapy.

Methods: The OS of 297 patients in a retrospective cohort is assessed. Multivariable statistical analysis is performed in order to establish the impact of the new markers on OS. The results of the analysis of the uPAR as well as the type IV collagen biomarkers are validated in a second cohort of 483 CRC patients and inclusion of CEA in the validation cohort is planned.

Result: The biomarkers were significantly associated to OS in univariable analyses with HRs (for a two-fold change in biomarker level) of 1.23 (95% CI: 1.15-1.32, p<0.0001) for CEA, 2.33 (95% CI: 1.79-3.04, p<0.0001) for suPAR(I-III), 2.91 (95% CI: 2.34-3.61, p<0.0001) for suPAR(I-III)+(II-III), 3.0 (95% CI: 2.35-3.83, p<0.0001) for uPAR(I) and 2.83 (95% CI: 2.27-3.53, p< 0.0001) for type IV collagen. The analysis showed that the only biomarkers retaining prognostic significance were uPAR(I) and type IV collagen in a reduced model with HRs of 1.92 (95% CI: 1.39-2.65, p<0.0001) and 1.69 (95% CI: 1.26-2.27, p=0.0005), respectively and a combined HR of 3.24 (95% CI: 2.44-4.30, p<0.0001) for these two markers. No significant interactions between the biomarkers and adjuvant chemotherapy could be demonstrated. Similar results were shown in the validation cohort.

Conclusions: High levels of uPAR(I) and type IV collagen were significantly associated to OS in the initial cohort outperforming CEA. Both uPAR(I) and type IV collagen demonstrated similar performance in the validation cohort. A predictive value, i.e. identifying patients benefitting from adjuvant chemotherapy could not be demonstrated.

Funding Source: Danish Research Group on Early Detection of Colorectal Cancer

Circulating Cell Free Nucleosome Levels Are Not Affected by Stage of CRC, Comorbidity or Demographic Variables

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Hypothesis: Are circulating cell-free nucleosomes (ccfn) containing methylated DNA (5mc), histone modifications (pH2AX, EZH2, H2AZ, H3K9Me3, H3K9Ac, H3K36Me3, H3S10Ph, H4K20Me3, H4PanAc), nucleosome adduct HMGB1 or total ccfn influenced by disease stage, comorbidity, and demographic variables in a study comparing individuals with colorectal cancer (CRC) to healthy individuals?

Objectives: Changes in levels of the ccfn have shown promising association for detection of CRC. However, the role of tumor burden, concomitant diseases, age, and gender has not yet been clarified. The present study evaluates the impact of these parameters on the ccfn in the discrimination of individuals undergoing diagnostic colonoscopy.

Methods: Levels of the ccfn and CEA were determined with NuQ® enzyme-linked immunosorbent assays in serum samples drawn from 4105 individuals undergoing diagnostic colonoscopy.
Linear modelling was used to assess the influence of disease stage, comorbidity, age, and gender. A predictor model was developed from a multivariate logistic regression model with cross-validated reduction based on levels of the ccfn and including CEA, age, and gender as explanatory variables; 5mc, pH2AX, and H3K36Me3 were included in the final model.

Result: Results are presented by the sensitivity, specificity, and the area under the receiver operating characteristic curve (AUC). Stage dependency (stage I-IV) of the ccfn levels could not be shown. A weak dependence on age with significantly decreasing levels at increasing age was shown for the ccfn ($R^2<0.02$). The level of H3S10Ph was the only ccfn showing a significant association to gender; all ccfn had $R^2<0.02$. Comorbidities (cardiovascular disease, rheumatic disease, lung disease, and diabetes) were weakly associated to the levels of the ccfn ($R^2<0.05$).
Results of the predictor model analysis showed similar results for individuals with CRC stage I+II (AUC=0.869) and stage III+IV (AUC=0.875) compared to individuals with clean colorectum.

Conclusions: Levels of the ccfn are independent of stage of disease, thereby improving the ability to detect individuals with early stage CRC. Furthermore, the influence of age, gender, and comorbidity is negligible.

Funding Source: Belgian Volition SA, Namur, Belgium

Colorectal Cancer Associated Biomarkers – The Effect of Bowel Preparation

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Hypothesis: Blood for determination of blood-based biomarkers for detection of colorectal neoplasia is often drawn at the time of colonoscopy. This may introduce biomarker result biases resulting from bowel preparation of the colonoscopy patients.

Objectives: To evaluate the influence of bowel preparation on protein and epigenetic biomarker levels in blood samples from subjects provided diagnostic colonoscopies.

Methods: Subjects (N=126) were recruited and blood samples collected before and after bowel preparation (immediately prior to colonoscopy). Concentrations of AFP, CA19-9, CEA, hs-CRP, CyFra21-1, ferritin, galectin-3, and TIMP-1 were determined in plasma using the Abbott ACHITECT® i2000 automated immunoassay platform. The epigenetic biomarkers including histone-modification H4K20m3, nucleosome-bound methylated DNA (5-mc) and total nucleosome levels were determined in serum with the Volition TECAN automated immunoassay platform. The galectin-3 ligand (an aberrantly glycosylated form of haptoglobin produced by neoplastic cells) was determined in serum using both a plate-based ELISA and a micro-bead platform.

Result: Pre-colonoscopy bowel preparation did not influence the levels of plasma AFP, CA19-9, CEA, hs-CRP or TIMP-1 (all p values >0.30), while the preparation had an insignificant reduction on plasma levels of Ferritin (p=0.07) and Galectin-3 (p=0.08). However, a highly significant decrease in the plasma levels of CyFra21-1 (28.7%; p<0.001) was shown from pre- to post bowel preparation. An association between the decrease in plasma CyFra21-1 levels and morbidity or co-morbidity could not be shown. Bowel preparation did not influence the levels of nucleosomes (all p-values >0.50) or galectin-3 ligand levels (p-values >0.80).

Conclusions: The present results demonstrated that pre-colonoscopy osmotic bowel preparation (Moviprep®) had a significant influence on the levels of plasma CyFra21-1, which decreased by 28.7%, but did not affect levels of eight other proteins and three nucleosomes.

Funding Source: N/A

Evaluation of Circulating Long Non-Coding RNA H19 and Cell-free Nucleosomes in Plasma of Patients with Gastric Cancer

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Hypothesis: Gastric cancer (GC) is one of the most common cancers and is thought to develop as a result of environmental factors and the accumulation of genetic and epigenetic alterations. Long non-coding RNAs (lncRNAs) are emerging molecules and have been shown to be involved in cancer development and progression. LncRNA H19 has been associated with GC. In recent years liquid biopsy draws a remarkable attention as a non-invasive procedure and is considered a potential source of the detection of cancer biomarkers. We hypothesize that the H19 in blood circulation could serve a biomarker in GC.

Objectives: In the present study we aimed to assess the impact of H19 and cell-free nucleosomes in GC. Fort this purpose, we measured plasma levels of H19 and nucleosomes at diagnosis of GC patients and compared to non-cancer individuals. We also evaluated these two markers after surgery of GC patients

Methods: Study participants consisted of patients with GC (N=38), individuals with gastric inflammation (N=24) and cancer- and inflammation-free control individuals (N=18), as verified by gastroscopy. Plasma samples were taken before gastroscopy for all participants and also after surgery (7-12 days) for a subset of GC patients (N=21). H19 was measured by real-time PCR while nucleosomes were determined by ELISA-based approach

Result: Our findings reveal that plasma levels of H19 did not differ between GC (median: 48,57) and control subjects (median: 93,05) (Mann-Whitney test, $p>0,05$) while significantly higher in GC patients compared to inflammatory disease (median: 6,34) ($p=0,006$). Comparing pre- and postoperative levels of H19 in GC, we detected significantly lower levels of H19 expression after tumor removal (median:15,15) (Wilcoxon Signed Ranks test, $p=0,002$). We found no significant differences in nucleosomes levels between study groups (Kruskal-Wallis, $p>0,05$). Nucleosomes levels did also not differ between pre- and postoperative plasma samples in GC patients (t test, $p>0,05$).

Conclusions: In summary, the results of this pilot study suggest that circulating H19 might be a potential marker in GC and warrant further research on this issue.

Funding Source: Istanbul University Research Fund (Project no: 53815)

Glad-PCR Assay of DNA Methylation Markers Associated with Colorectal Cancer

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Hypothesis: Colorectal cancer (CRC) is one of the major malignancies leading to a high incidence and cancer death worldwide. Hypermethylation of the gene regulatory regions is shown for many cancer diseases including CRC. The detection of epigenetic biomarkers is one of the most promising diagnostic and prognostic tools. On the basis of recently discovered unique methyl-directed site-specific DNA endonuclease GlaI we developed a GLAD-PCR assay (GlaI hydrolysis and Ligation Adapter Dependent PCR) allowing quick and inexpensive estimation of 5’-R(5mC)GY-3’ site in a definite position of human genome without bisulfite conversion.

Objectives: It is known that an aberrant DNA methylation in cancer cells is catalyzed by DNA methyltransferases Dnmt3a and Dnmt3b, which predominantly recognize and methylate RCGY sequences with formation of R(5mC)GY sites. Recently, based on a new methyl-directed DNA endonuclease GlaI, we developed a GLAD-PCR assay, which allows to determine R(5mC)GY site in a definite position of the genomic DNA. In this work we have applied GLAD-PCR assay for identification of the methylated RCGY sites in the regulatory regions of some downregulated genes associated with colorectal cancer (CRC). This list includes ADHFE1, ALX4, CNRIP1, EID3, ELMO1, ESR1, FBN1, HLTF, LAMA1, NEUROG1, NGFR, RARB, RXRG, RYR2, SDC2, SEPT9, SFRP2, SOCS3, SOX17, THBD, TMEFF2, UCHL1 and VIM genes. GLAD-PCR analysis of selected RCGY sites within the regulatory regions of some of these genes demonstrates a good prognostic potential with relatively high sensitivity and specificity of CRC detection in tumor DNA.

Methods: Briefly, GLAD-PCR assay is carried out in three steps. At the first step GlaI cleaves R(5mC)GY sites in genomic DNA. Then the obtained DNA fragments are ligated with the unique oligonucleotide adapter. The last step is a PCR with TaqMan probe and a genomic primer, which are complementary to target DNA fragment, and a so-called hybrid primer, which is complementary to the adapter and partially to the genomic sequence at the cleavage point of the site of interest. As a result, despite of the presence of a huge number of different DNA fragments obtained after GlaI hydrolysis and a ligation step, PCR takes place specifically from the target region of DNA.

Result: GLAD PCR assay of DNA from a colorectal adenocarcinoma cell line SW837 was used to reveal R(5mC)GY sites in selected regions of 23 genes (26 studied regulation regions in total) where sensitivity and specificity have been determined for each site. Tissue samples (n=21) of colorectal adenocarcinomas of varying degree of differentiation and 9 paired normal colon mucosa samples were studied by GLAD-PCR assay. The data revealed a high level of methylation in the selected methylated sites among all tumor showing maximal number of unmethylated RCGY site in HLTF and SDC2 regulation regions. At the same time GLAD PCR assay showed an absence of the methylation of the selected RCGY sites for SDC2, FBN1(3.3), FBN1(3.1), SEPT9, THBD and VIM genes. Surprisingly, we observe methylation of selected RCGY sites in all normal samples in case of TMEFF2 and SOX17 regulation regions and in the most of normal samples in EID3 and ESR1 genes.

Conclusions: In this study we have applied a new GLAD-PCR assay to identify an aberrantly de novo methylated RCGY sites in several downregulated genes in CRC. The analysis of their methylation status demonstrated a good prognostic potential with relatively high sensitivity and specificity of CRC detection in the tissue DNAs. Moreover, we suppose that these sites may serve as suitable predictive markers for noninvasive blood and stool screening for CRC by GLAD-PCR as well.

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Immunotherapy in Endocrine Dependent Metastatic Breast Cancer

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Hypothesis: Hormonetherapy is advised for ER+ metastatic breast cancer patients because of its efficacy concomitant with low toxicity, however in most patients the occurrence of resistance is a not yet well understood hurdle to overcome.
In these patients during clinical benefit from conventional antiestrogens the addition of cycles of sequential immunotherapy could prolong the benefit and delay the arising of acquired hormone resistance.

Objectives: In order to validate this hypothesis in 1992 we started an open exploratory clinical trial and more times during the study we have reported on results and the potential rationale. They both are here updated.

Methods: Forty-two operated breast cancer patients with distant metastases and in clinical benefit during first line antiestrogen salvage therapy were recruited. Beta-interferon (INF beta) 3,000.000 IU im/day 3 days/week, weeks 1-4 and successively recombinant interleukin-2 (IL-2) 3,000.000 IU sc/day 3 days/week, weeks 5-8 were added to antiestrogens until to progression. The immunotherapy cycle lasted 10 weeks and the patient continued antiestrogens only during weeks 9-10, the 11th week being the first week of the successive cycle.

Result: The pattern of laboratory data showed an immune stimulation during clinical benefit and that during clinical benefit from combined hormone-immunotherapy differently than at the progression no relevant immune inhibition occurred. The addition of INFbeta-IL-2 sequence significantly prolonged clinical benefit and overall survival from conventional antiestrogens.

Conclusions: To further confirm these promising results of a more general immunological approach to delay acquired hormone resistance a multicenter prospective phase II trial is going to be launched by the Cancer Center Institute of Tuscany in Italy.

Funding Source: N/A

Selected Growth Factors and Different Gastric Neoplasms in Humans

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Hypothesis: Abnormal interactions between various growth factors may be an overlooked mechanism linking the development of different types of gastric neoplasms in humans. Therefore, in this study we i)compared levels of selected growth factors among patients with gastric neoplasms and healthy volunteers; ii)verified the potential associations between systemic levels of examined substances and staging of gastric cancer; and iii)estimated potential diagnostic benefits that can be derived from measurements of systemic levels of growth factors in patients with lesions detected within the gastric tissue.

Objectives: For this study 75 patients with gastric neoplasms (cancer, gastrointestinal stromal tumors, neuroendocrine neoplasms, lymphomas) and 40 healthy volunteers were included.

Methods: Systemic levels of hepatocyte, fibroblast and vascular-endothelial growth factors (HGF, FGF, and VEGF, respectively), as well as, of insulin-like growth factor-1 (IGF-1) were measured using commercially available ELISA tests.

Result: The results obtained demonstrated, that patients with gastric cancer have significantly higher systemic levels of HGF, FGF and VEGF, as well as lower concentrations of IGF-1, in comparison to both healthy volunteers and patients diagnosed with other types of gastric neoplasms ($p<0.05$ for all). In patients with gastric cancer systemic levels of only HGF significantly correlated with cancer staging evaluated according to the TNM classification. Based on receiver operating characteristic curve analysis systemic levels of examined growth factors did not appear to hold diagnostic potential in confirming or excluding the presence of gastric cancer in humans (area under curve values of 0.48-0.65, and $p>0.08$ for all).

Conclusions: In patients diagnosed with gastric cancer, an abnormal systemic biochemical balance in multiple growth factors occurs. This phenomenon exists as soon as on the earlies stages of gastric cancer development in humans and seems to be specific only for gastric cancer. Furthermore, systemic levels of examined growth factors do not seem to possess sufficient diagnostic value to be used as independent markers of gastric cancer in humans.

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Significance Estimation of EGFR and Their Polymorphisms -216G>T (rs712829), -191C>A (rs712830) and 181946C>T (D994D) (rs2293347) in Non-Small Cell Lung Cancer

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Hypothesis: Epidermal growth factor receptor (EGFR) was usually over expressed in many epithelial cancers including lung cancer. It is trigger molecule for many important processes in normal cells concerning growth, development, differentiation, but in tumor cells it conduct many abnormal messages through signaling network cascade leading to cancer genesis.

Objectives: Genetic and epigenetic changes in lung cancer, including single nucleotide polymorphisms (SNPs) are in the course of scientific interest with many reasons.

Methods: Here we described methods for standardization of PCR reaction including different additives, temperature and other conditions, for estimation of expression of promoter regulators of EGFR, including -216G>T, -191C>A and 181946 C>T (SNPs) in 47 NSCLC patients in comparison to 43 health persons in Serbia. These data where compared with results obtained in different ethnic populations.

Result: Significant differences in EGFR and SNPs distributions were noticed through great ethnic populations. These results showed that the most frequent haplotypes in both NSCLC patients and healthy subjects in Serbia were CG (54.96%), CT (27.36%), while AG (16.38%) and AT where present at 1.30%. Caucasians and Afro-Americans had more frequent -216G/T than Asians, but -191C/A was present only in Caucasians. SNPs -216G>T and -191C>A discussed here, were present with different frequency in great ethnic groups.

Conclusions: Still it is unclear relation between those polymorphisms and common mutations, but they were connected with enhanced EGFR promoter activity, with increased gene and protein expression, and some side effects of TKI used for NSCLC. According to those considerations, it could be noticed that investigation of SNPs could be potential pharmacogenetic biomarker for efficacy and safety of TKI treatment approach for NSCLC.

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Reactive Oxygen Species in Non-Hodgkin's Lymphoma patients

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Hypothesis: Non-Hodgkin`s lymphoma (NHL) is a heterogeneous group of clonal, proliferative, abnormal diseases of B- or T-cells and is usually first diagnosed in lymph nodes or as extranodal lymphatic masses. The generation of free radicals and ROS contributes to a broad spectrum of normal physiological processes, while excess of ROS results in DNA and protein damage and consequtive participated in cancerogenesis.

Objectives: Identificatoin of the effects of excess reactive oxygen species on DNA, protein damage, and consequtive cancerogenesis.

Methods: In this study, 32 patients with NHL and 13 healthy volunteers were studied. The diagnosis of NHL patients was based on histological documentation of lymph node and/or bone marrow biopsies. Clinical staging was performed according to the Ann Arbor system based on combination of clinical routine examination, blood and ultrasound analyses, chest ray and computerized tomography (CT) scan. The pathohistological evaluations for 32 patients, aged from 27 to 57 yr, with NHL was made according to the International Working Formulation, and IPI score index. Superoxide-anion radical and TBARS , and GSH (glutathione extraction) are determined by biochemical methods previously establisched.

Results: A statisticaly significant ($p<0.05$) increase of levels of O2 was measured in patients who were in advanced clinical stage IV. The levels of TBARS, as well as GSH, increased in plasma of NHL patients and correlated with increased clinical stage ($p<0.05$, ANOVA). The generation of O2.-was significantly higher ($p<0.05$) in plasma of NHL patients with high grade of malignancy as compared to the controls. There were no differences in the values in patients with low or intermediate grade of malignance. The results showed significantly increased levels of TBARS ($p<0.05$, ANOVA) in plasma of NHL patients in all three histological types as compared with controls. Plasma levels of GSH, were significantly increased ($p<0.05$, ANOVA) in NHL patients as compared with controls in all histological types. There were no differences for these parameters between the histological types of NHL patients.

Conclusions: The abnormal production of cellular oxidants or the imbalance of the oxidant to the antioxidant control systems have been linked to mutation and by oxidant -induced DNA damage and many other changes in gene expression. Moreover, several signal transduction pathways, such as activator protein-1 and Nuclear Factor-Kb (NF-Kb), are known to be activated by ROS. This leads to the transcription of genes involved in cell growth regulatory pathways. Based on this estimation of these parameters in serum of patients with cancer can help in diseases monitoring.

Funding Source: N/A

Are Circulating Growth Factors Suitable for Predicting Lymph Node Metastases and for Clinical Decision About Axillary Lymph Node Dissection in Early Breast Cancer Patients?

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Hypothesis: Growth factors actively participate in tumor-induced angiogenesis and lymphangiogenesis and their serum levels may reflect lymph node (LN) metastases formation in early breast cancer.

Objectives: Lymph node metastases are one of risk factor in early stage breast cancers and their presence determine the extent of surgical intervention. Axillary lymph node dissection (ALND) is a standard procedure, however a number of studies provided evidence that avoiding ALDN does not worsen the general prognosis. The aims of our study were to evaluate the possibility of selected growth factors as biomarkers for lymph node status determination and for clinical decision as regards avoiding ALND in early breast cancer patients.

Methods: 211 patients with malignant breast cancer and 42 age-matched breast cancer-free controls were enrolled in the study. 78 patients had one or more lymph node metastases; in 31 LN positive patients with low risk tumor ALND was avoided. Levels of insulin-like growth factor 1 (IGF1), insulin-like growth factor binding protein 3 (IGFBP3), epidermal growth factor (EGF) and vascular endothelial growth factor (VEGF) were measured in serum and plasma by immunoanalytical techniques. We compared levels in patients with negative vs. positive lymph node status and in patients with and without axillary lymph node dissection.

Results: We found significantly elevated serum IGF1 and plasma VEGF levels in patients with lymph node metastases compared to controls (p=0.0179 and p=0.0091, respectively) and in patients which underwent axillary lymph node dissection (p=0.0337 and p=0.0438).

Conclusions: Circulating IGF1 and VEGF levels may predict the presence of lymph node metastases and help in clinical decision to avoid axillary lymph node dissection in patients with early breast cancer.

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Expression and Epigenetic Regulation of the Phospholipase A2 Receptor in Mammary Cancer Cells

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Hypothesis: It has recently been proposed that the M-type phospholipase A2 receptor (PLA2R1) acts as a tumor suppressor in certain malignancies including mammary cancer.

Objectives: Considering that DNA methylation is an important regulator of gene transcription during carcinogenesis, in the current study we analyzed the PLA2R1 expression, PLA2R1 promoter methylation, and selected micro RNA (miRNA) levels in normal human mammary epithelial cells (HMEC) and cancer cell lines.

Methods: Levels of PLA2R1 and DNA methyltransferases (DNMT) specific mRNA were determined using real-time RT-PCR. Methylation specific-high resolution melting (MS-HRM) analysis was utilized to quantify the methylation degree of selected CpG sites localized in the promoter region of the PLA2R1 gene. Expression of miRNA was tested using miScript Primer Assay system.

Result: Nearly complete methylation of the analyzed PLA2R1 promoter region along with PLA2R1 gene silencing was identified in MDA-MB-453 mammary cancer cells. In MCF-7 and BT-474 mammary cancer cell lines, a higher DNA methylation degree and reduced PLA2R1 expression were found in comparison with those in normal HMEC. Synergistic effects of 5-aza-2´-deoxycytidine and trichostatin A on PLA2R1 transcription in MDA-MB-453 cells confirmed the importance of DNA methylation and histone modification in the regulation of the PLA2R1 gene expression in mammary cells. Furthermore, significant positive correlation between the expression of DNMT1 and PLA2R1 gene methylation and negative correlation between the cellular levels of hsa-mir-141, -181b, and -181d-1 and the expression of PLA2R1 were identified in the analyzed cells. Analysis of combined z-score of miR-23b, -154 and -302d demonstrated significant positive correlation with PLA2R1 expression.

Conclusions: Our data indicate that (i) PLA2R1 expression in breast cancer cells is controlled by DNA methylation and histone modifications, (ii) hypermethylation of the PLA2R1 promoter region is associated with up-regulation of DNMT1, and (iii) hsa-miR-23b, -154, and -302d, as well as hsa-miR-141, -181b, and -181d-1 are potential candidates for post-transcriptional regulation of PLA2R1 expression in mammary cancer cells.

Funding Source: N/A

IGF1R Blockades for Gastrointestinal Cancer Cells with Mutated Kras Gene

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Hypothesis: kRAS mutation plays important roles in both cancer progression and resistance to anti-EGFR therapy in GI tumors. IGF1R blockade might be effective for kRAS-MT overexpressing GI cancer.

Objectives: To evaluate effects of kRAS-MT expression on GI cancer cell lines representing a possible second resistance mechanism for antiEGFR therapy and IGF1R targeted therapy for these transfectants.

Methods: We made stable transfectants of kRAS-MT in 2 GI cancer cell lines. We assessed the effect of overexpression of kRAS-MT. We assessed antitumor effects of dominant negative and an IGF1R inhibitor.

Result: kRAS-MT expression in GI cancer cells led to more aggressive phenotypes. IGF1R blockade showed anti-tumor effects even when kRAS-MT was overexpressed, both in vitro and in murine xenografts.

Conclusions: kRAS-MT might be important for progressive phonotype observed in GI cancers. IGF1R targeting therapy is a candidate molecular therapeutic approach for GI cancers even if kRAS is mutated.

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Anti-Tumor Effect of Anti-erbB-2 Trifunctional Antibody

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Hypothesis: Erb-B-2, a member of the EGF receptor tyrosine kinase family, is often overexpressed and/or amplified in breast cancer, gastric cancer and other malignancies.

Objectives: We established an anti-erbB-2 mouse-human chimeric monoclonal antibody (MoAb) CH401, since erb-B-2 gene product is one of the best target molecules for cancer therapy. The MoAb CH401 is able to kill erbB-2 positive cancer cells in vitro, we investigated the mechanism of tumor suppression by MoAb CH401 and its therapeutic usefulness. Furthermore, we tried to produce the anti-erbB-2 and anti-CD3 trifunctional antibody for seeking more powerful anti-tumor activity.

Methods: Western blot, flow cytometric and immunocytochemical analysis were performed to investigate the mechanism of tumor suppression by MoAb CH401 and its therapeutic application. In addition, we studied the efficacy of anti-erbB-2 and anti-CD3 trifunctional antibody in vitro.

Result: It is found that anti-erbB-2 mouse-human chimeric monoclonal antibody CH401 induces apoptosis in erb-B2 overexpressing cells by activating the JNK/p38 pathway and down-regulating the ERK pathway. We established the anti-erbB-2 and anti-CD3 trifunctional antibody and revealed its tumor suppression effect.

Conclusions: These results suggest that the anti-erbB-2 and anti-CD3 trifunctional antibody will be a promising approach to the treatment of erbB-2 expressing cancer cells. And this trifunctional antibody is shown to have an effective potential for Herceptin resistant cancer cells.

Funding Source: MEXT of Japan

The Role of Human DNA Repair Protein APE1/Ref-1 In Transcriptional Regulation of Genes Involved in Cancer Development.

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Hypothesis: Apurinic/apyrimidinic endonuclease 1, also named redox effector factor 1 (APE1/Ref-1), is a DNA repair protein which stimulates binding of transcription factors (TFs) to the DNA. APE1/Ref-1 – dependent stimulation of TFs may lead to activation of angiogenesis, anaerobic metabolism, hypoxia, cell adhesion and cell growth, causing tumor resistance to anti – cancer treatments. APE1/Ref-1 can be the promising target to sensitize tumor to anti – cancer treatments. The mechanism how APE1/Ref-1 stimulates TFs binding to the DNA is not clearly understood. We hypothesize that APE1/Ref-1 enzyme activates TFs by direct interactions with the DNA via amino acids located in N-terminal domain.

Objectives: The aim of the presented work is to perform mechanistic and biochemical studies on the contribution of N-terminal domain of APE1/Ref-1 enzyme in the stimulation of TFs.

Methods: By the use of Western analysis we studied the level of APE1/Ref-1 protein in HeLa cells, resistant and sensitive to photodynamic therapy (PDT). Using the Electrophoretic Mobility Shift Assay (EMSA) we compared how APE1/Ref-1 wild – type (wt), APE1/Ref-1 deleted from the N-terminal domain and APE1/Ref-1 deficient in nucleotide incision repair activity stimulate binding of TFs to the DNA. Using enzymatic assays we studied how deletion of N-terminal domain influences DNA repair activities of APE1/Ref-1. By the use of Surface Plasmon Resonance imaging and EMSA approaches we compared how APE1/Ref-1 wt and APE1/Ref-1 mutant proteins interact with the DNA. Using EMSA we compared how APE1/Ref-1 stimulates binding of TFs to different DNA conformations that APE1/Ref-1 interacts or does not interact with.

Result: Following photodynamic therapy (PDT) the level of APE1/Ref-1 significantly increases in HeLa cells PDT resistant, which is not the case for the sensitive cell line. APE1/Ref-1 wt and APE1/Ref-1 deficient in nucleotide incision repair activity stimulate oligomerization of TFs on the DNA, which is not the case for APE1/Ref-1 deleted from the N-terminal domain. APE1/Ref-1 acts synergistically to reducing agents during stimulation of TFs on short oligodeoxynucleotides. Deletion of N-terminal domain significantly decreases APE1/Ref-1 – dependent stimulation of DNA glycosylases, impairs nucleotide incision repair activity and reduces strong interaction with single – stranded DNA. APE1/Ref-1 fails to stimulate oligomerization of TFs on the DNA conformations that the enzyme cannot interact with.

Conclusions: The mechanism how APE1/Ref-1 stimulates binding of TFs to the DNA can be explained by direct interactions between N-terminal domain of APE1/Ref-1 and the DNA. We conclude that APE1/Ref-1 acts as DNA-chaperone protein, which induces changes on DNA conformation to stimulate binding of TFs to the DNA.

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Prototype Biomarker Panel Algorithms for Aiding in Early Colorectal Cancer (CRC) Detection

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Hypothesis: Analyses of blood-based cell-free protein biomarkers may aid in screening identification of subjects that should be offered subsequent to colonoscopy.

Objectives: The present study was to develop proof-of-concept blood based biomarker algorithms to aid in early detection of CRCs. A primary screen has been developed to detect CRCs/high-risk adenoma lesions. A secondary/reflex algorithm has been developed to assess those false positive subjects from the primary CRC screen for the presence of other extra-colonic cancers as the primary screen markers may not be CRC-specific.

Methods: CRC screening trial Endoscopy II included 399 high-risk adenomas and 512 CRCs from a study population of 4,698 high risk symptomatic subjects. Trial plasma specimens were assessed on the Abbott ARCHITECT system for CEA, CA19-9, Cyfra 21-1, AFP, Galectin-3, TIMP-1, hs-CRP, & Ferritin. In addition, for the reflex algorithm, 10 additional markers were tested including SCC, ProGRP, CA125, HE4, B2M, TPSA, CA15-3, Pepsinogen I & II, and NSE. Algorithms of optimized biomarker subsets with cutoffs were generated and cross-validated using an Adaptive Index Model (AIM) for both the primary and reflex algorithms.

Result: Sex-specific algorithms to detect CRCs/high-risk adenoma patients included four biomarkers (CEA, Cyfra 21-1, hsCRP, Ferritin) and age. A positive signature model (≥2 factors meeting the cutoff) had sensitivity and specificity of 55% and 81% for men, and 62% and 67% for women. A sex-specific reflex algorithm to address risk of extra-colonic cancer in primary screen colonoscopy negative subjects was improved over previous pilot algorithms by including additional markers in the AIM algorithm.

Conclusions: These data support that multivariable algorithms can yield useful discrimination between non-cancer subjects and those having high-risk adenomas or CRC, as well as addressing risk of extra-colonic cancer/malignancy in false positives for primary screen. Algorithm enhancements continue to be explored by inclusion of other strong biomarker leads from the CRC early detection field, and by refinement of algorithm criteria.

Funding Source: The Danish Research Group on Early Detection of Colorectal Cancer, and Abbott Laboratories

The Potential Role of DKK-1&B-Catenin as Biomarkers for Diagnosis of HCC

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Hypothesis: The present work was to investigate serum Wnt/B-catenin &DKK1 levels can be used to predict the progression of CHC into HCC at earlier stages.

Objectives: Study the role of DKK1 &B-catenin as biomarkers for diagnosis of HCC

Methods: A total of 150 patients with CHC were divided into 3 groups HCV without cirrhosis CHC, patients with cirrhotic liver LC &HCC. Liver function, HBs Ag HCV antibodies, AFP,ALF-L3,DKK1,B-catenin .

Result:DKK1were significantly increased p less0.001 in HCC 324.2+-94.6 from
both LC 229.9+-52.7 &CHC 180.7+-37.3, B-catenin were significantly increased p less 0.001 in HCC

Conclusions: DKK1 and B-catenin potentially may be used as predictors for progression of CHC and LC into HCC.

Funding Source: National Hepatology &Tropical Medicine Research Institute

Lung Metastasis from Metastasizing Pleomorphic Adenoma of Submandibular Gland

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Hypothesis: Hypothesis: Pleomorphic adenomas (PA) are the most common benign salivary gland tumors, although, only, about 10% of them occur in the submandibular gland. Metastasizing pleomorphic adenoma (MPA) constitutes an extremely rare group of tumors.
MPA has a slight higher incidence in females in the third decade of life. The average latency time between the diagnosis of the PA and the discovery of metastases is about 16 years. Most MPA have at least one local recurrence at the primary site. Mortality ranged varies from 20% to 40%. Patients presenting with metastasis within 10 years of the occurrence of their initial primary tumor have a worse prognosis.

Objectives: Describe a very rare clinical case of pulmonary metastases originating from a salivary gland adenoma.

Methods: Case report: A 75-year-old man, smoker, with chronic obstructive pulmonary disease, hypercholesterolemia and essential hypertension. He was diagnosed with PA of submandibular gland; six years later, he had a local recurrence. The primary tumor and the recurrence did not show features of malignancy.

Result: After eight months, the patient presented an incidental finding of multiple bilateral lung shadows on chest radiography. The computerized tomography of abdomen and thorax identified multiple nodules in both lungs. The histologic examination showed an admixture of small cords, trabeculae and glandular foci in a chondromyxoid stroma. No histological features suggestive of malignancy were observed. The cells had bland, round to ovoid nuclei and were positive to S-100, actin and p63 and negative to TTF-1. The clinical and histologic data rendered a diagnosis of MPA. Tumor markers, such as AFP, PSA, CEA, CA 125, CA 15.3 and CA 19.9, were within normal limits. Nowadays, the patient remains stable.

Conclusions: In our case, the MPA occurred in a man older than the average age of these patients; furthermore, the period between the diagnosis of the primary tumor, the recurrence and metastases was relatively short. Perhaps, in older patients some degree of immunosuppresion facilitates the metastatic spread.
Nowadays, there are no histopatological immunohistochemical or serological markers that can differentiate a MPA from a PA.

Funding Source: N/A

Early Diagnosis of Advanced Colorectal Neoplasia by Fecal Occult Blood Test in an Elevated Risk Population

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Hypothesis: First degree relatives of patients with colorectal cancer (CRC) have a 2.5-fold to 3-fold increased risk of developing CRC. Colonoscopy is the gold standard for advanced colorectal neoplasia (ACN) diagnosis. The ACN condition includes the CRC and the advanced adenomas (lesions with one of those three conditions: high-grade dysplasia or size over 10 mm or presence of a villous component). However, colonoscopy has several important limitations such as possible serious complications, patient discomfort, low acceptance and high cost. Fecal occult blood test (FOBT) has been recommended as method of screening for CRC but there are little reports in elevated risk population.

Objectives: Determine the diagnostic utility of FOBT for ACN early detection in an elevated risk population.

Methods: The study included 338 asymptomatic first degree relatives (parents, siblings or offspring) of patients with CRC. The mean follow-up of patients was 36.9 months (SD 14.2 months). All patients underwent screening FOBT and colonoscopy. Fecal samples, which were collected before colonoscopies, were analyzed using the fully automated analyzer OC-SENSOR (EIKEN CHEMICAL, Tokyo, Japan); results are expressed in µg/g feces. The selection criteria excluded those patients with personal history of CRC, colonic adenomas and Lynch syndrome.

Result: Three hundred thirty-eight relatives (195 females, 57.7%), 51.7±9.7 (range, 30–75) years of age, agreed to participate out of a total of 794 who were invited (acceptance rate 42.5%). ACN was found in 58 (17.2%) participants. Of these, CRC cancer, CRC on polyp and advanced adenomas were detected in one (0.3%), three (0.9%) and 54 (15.98%) participants, respectively. Low-risk adenomas, hyperplasic polyps and unidentified polyps were found in 8 (2.37%), 25 (7.40%) and 10 (2.96%) subjects, respectively. In 237 cases colonoscopy was normal. The ROC AUC (95% CI) for ACN detection by FOBT was 0.733 (0.662–0.804). A 20.5 µg/g FOBT cut-off showed a sensitivity of 44.8%, a specificity of 84.3% and a predictive negative value of 91.57%.

Conclusions: FOBT screening of first-degree relatives of patients diagnosed with CRC allow the detection of ACN and an effective classification of high and low risk subjects.

Funding Source: N/A

High Unnecessary Costs Caused by Slight Increase of CEA Levels

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Hypothesis: Carcinoembryonic antigen (CEA) could be increase in patients without cancer due to processes that increment the release or reduce catabolism, and so give rise to false positives. In these cases, differential diagnosis with other pathologies may require a large number of tests. Complementary studies derived by a slight elevation of CEA, without proper interpretation and handling of this result, can generate important economic spending.

Objectives: To determine the prevalence of cancer processes in patients with positive low CEA levels, the causes of false positives found, and the cost for the health system study them.

Methods: Retrospective observational study (seven years) of all patients over 18 years with a slight elevation of CEA (3-10 ng/mL), with no history of cancer, who were referred to gastroenterology from primary care or other services to a tumor screening process. All the patients underwent a mean of 54.3 months (12-132) after the study CEA levels were analyzed using a cobas® 6000 (Roche Diagnostics).

Result: A total of 187 medical records were reviewed. 87 patients were excluded for lack of clinical data in history, lack of evolutionary study or for smoking with CEA levels of 3-5 ng/mL. One hundred patients were included (40% females, age range, 23-95). Four cases with neoplasia were detected (stomach, colon and two lung cases) after the initial study and three during monitoring (larynx and acute myeloid leukemia with CEA decreasing levels; colon, with CEA increasing levels). In 45 patients were found false positive causes (16 chronic lung disease, 12 renal failures, 6 cirrhosis, 3 pancreatitis, 2 adenomas, and 2 primary hypothyroidism). In the other 48 cases no cause to justify raising the marker was detected. Only serial CEA determinations were performed on 53 patients, but with inadequate intervals (range 2- 70 months). The average cost per patient generated by the complementary studies for the detection of a possible hidden tumor in relation to the elevation of CEA was 503.6±257.6 €.

Conclusions: Identifying the causes of a low increase CEA levels in the absence of cancer (false positive) is vital to the correct interpretation of tumor marker results.

Funding Source: N/A

A Novel Serological Marker for Pancreatic Cancer

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Hypothesis: A glycoprotein which we found in condition media of cultured human pancreatic ductal adenocarcinoma (PDAC) cells may be useful as a serum diagnostic marker for PDAC.

Objectives: Accurate peripheral markers for the diagnosis of PDAC are lacking. Biomarkers for the diagnosis of patients with PDAC are needed to improve prognosis.

Methods: A case-control study included two subcohorts: the discovery cohort that included 23 PDAC patients and 51 control individuals, and the validation cohort that included 29 PDAC patients and 14 controls.

Result: The discovery cohort demonstrated that the AUC were 0.973 (95%CI 0.943-1) for this glycoprotein and 0.802 (95%CI 0.693-0.912) for CA19-9. The validation cohort showed the similar results.

Conclusions: Our data suggest that measuring the level of this glycoprotein has the potential to improve detection of PDAC. Further research is necessary to confirm its value for early detection of PDAC.

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An Anti-PSMA Immunotoxin Induces Apoptosis and Efficiently Eliminates Prostate Cancer Cells in Combination with ABT-737

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Hypothesis: Anti-apoptotic Bcl-2 proteins are overexpressed in prostate cancer. They deregulate the cellular death pathway and thus promote cancer cell survival and chemoresistance. Therefore, anti-apoptotic Bcl-2 downregulation or inhibition might be a promising approach for treatment and control of chemoresistance in prostate cancer.

Objectives: Due to the fact that there is still an enormous complexity of treating advanced prostate cancer, we adopted the strategy to directly induce intrinsic apoptosis on the level of pro- and anti-apoptotic proteins in prostate cancer cells.

Methods: We developed a recombinant immunotoxin that specifically binds to the prostate specific membrane antigen (PSMA) and possesses the toxic fragment of Pseudomonas aeruginosa Exotoxin A (PE 40). Androgen-sensitive (LNCaP) and androgen-independent (C4-2) prostate cancer cells were treated with the immunotoxin alone or in combination with the BH3-only mimetic ABT-737. ABT-737 is known to effectively inhibit the anti-apoptotic proteins Bcl-2, Bcl-xl, and Bcl-w, but not Mcl-1. We scrutinized the activation of apoptotic pathways and anti-tumor effects by Western blotting and WST-1 cell based cytotoxic assay.

Result: We could demonstrate a downregulation of Mcl-1 by the immunotoxin followed by induction of caspase-3 and cleavage of poly (ADP-ribose) polymerase (PARP). Administration of the anti-PSMA immunotoxin resulted in substantial cytotoxicity with 50% reduction of cancer cells (IC 50) in the low nM range. The cytotoxicity of ABT-737 was clearly weaker with IC 50 values in the µM range. Remarkably, simultaneous application of subtoxic concentrations of immunotoxin and ABT-737 resulted in significantly enhanced cell death.

Conclusions: This work substatiates that immunotoxin-mediated apoptotic action is enhanced by inhibition of anti-apoptotic proteins of the Bcl-2 family with ABT-737. Thus anti-PSMA immunotoxin / ABT-737 combination could deliver an effective strategy for the treatment of advanced prostate cancer.

Funding Source: N/A

Diagnostic Value of Interleukin-31 and 33 in Endometrial Cancer

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Hypothesis: Interlukin-31 and Interlukin-33 may be potential tumor markers for endometrial cancer (EC)

Objectives: Previous evidence proved that interleukin-31 and 33 can be potential tumor markers in some cancer's formulation. We aimed to determine the potential role of them in prognosis of endometrial cancer

Methods: Serum samples collected from 160 EC patients and 160 healthy controls and were tested by ELISA kits. Serum levels of tumor markers were tested by chemiluminescence immunoassay.

Result: Serum levels of IL-31 and 33 in patients were also related to including tumor stages, depth of invasion, node and distant metastases. The sensitivity and specificity were higher than tumor markers.

Conclusions: This is the first research mentioned the possible association between serum IL-31 and 33 with EC. With its sensitivity and specificity, the interleukins may be useful biomarkers for EC‘s diagnosis.

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Plasma cfDNA for Early Cancer Detection

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Hypothesis: The presence of circulating cell-free DNA (cfDNA) from tumours in blood is of major importance to those interested in early cancer detection, as well as those wishing to monitor tumour progression or diagnose the presence of activating mutations to guide treatment.

Objectives: Our aim was to review the circulating tumour-derived DNA (ctDNA) literature to examine the best options for the detection of multiple multiple cancer types.

Methods: Comprehensive searches of electronic search databases Medline, Embase, CINAHL, the Cochrane library, and Biosis to obtain relevant literature on blood-based biomarkers for cancer detection in humans (PROSPERO no. CRD42014010827). The abstracts for each paper were reviewed to determine whether validation data was included, and then examined in full. Papers concentrating on monitoring of disease burden or mutations were excluded.

Result: The initial mapping review identified a list of 70 cfDNA markers. Plasma was used for 34 markers, and serum for 23 markers, and either for 13 markers. Only two comparative studies of serum and plasma were conducted: one for BRAF mutations, and the other for PIK3CA mutations. Assays were categorised as polymerase chain reaction (PCR), real-time quantitative polymerase chain reaction (qPCR), digital droplet PCR (ddPCR), BEAMing, or next generation sequencing (NGS). The vast majority of studies (n = 54) used PCR, usually real-time PCR, and 31 studies examined methylation. Sequencing was used in 13 studies, and NGS was noted to allow the measurement of large numbers of mutations with sufficient sensitivity.

Conclusions: We have systematically reviewed cfDNA blood biomarkers for the early detection of cancer. A number of pre-analytical, analytical, and post-analytical considerations were identified which need to be addressed before such biomarkers could enter clinical practice.

Funding Source: Cancer Research UK, London, UK.

Biomarkers –Best Assays to Assess Response to New Therapies and Provide Prognosis in Melanoma Patients

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Hypothesis: Malignant Melanoma's incidence is increasing during last years, while mortality is strongly decreasing due to improved early detection, close monitoring of patients including Biomarkers and introduction of new therapies.

Objectives: The aim of the present study was to evaluate a panel of Biomarkers, (S-100b, OPN, MIA, sIL-2R, TK) in Melanoma patients, as to their ability to assess treatment response, especially to introduction of new therapies.

Methods: We evaluated both retrospectively and prospectively 197 Malignant Melanoma patients. Blood Biomarker levels were evaluated by conventional ELISA assays. Correlations of marker levels to disease stage, metastases, response to new immunotherapies and survival, were performed.

Result: Serum levels of Biomarkers were significantly higher in all patients before various therapies were applied, n=79, (5.35+0.7) and decreased thereafter, n=56 (1.4+0.3). Significantly higher levels of S-100β were demonstrated in advanced disease including Metastases, (6.97+0.52) as opposed to early disease (0.32+0.07) and NED patients. (0.16+0.04). When comparing Melanoma deceased patients who had extremely high levels of S-100β, (4.2+0.35) we showed significantly lower levels in alive patients (0.26+0.02) and certainly in normal controls. (0.08+0.02). In individual patients, kinetic evaluations showed earlier the response to therapy, or recurrence and non-response, as shown only after a few months later, by CTs evaluations

Conclusions: S-100β and most of the other tested biomarkers, can serve as the most useful Biomarker for assessment of treatment response and prognosis, especially after using new immunological treatments as Anti BRAF, IPI or Anti PD1 in Malignant Melanoma patients. Additional biomarkers as LDH, b2M, sIL-2R and TK, may also serve as part of a biomarkers panel, for improved detection of recurrence and metastasis or treatment changes, affecting survival.

Funding Source: Laboratory Fund

The Diagnostic and Prognostic Value of Sil-2R as an Immune Biomarker in Head and Neck Cancers

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Hypothesis: Head and Neck cancer (HNC) patients are diagnosed with usually advanced disease and multimodality therapies are required, as well as prognostic biomarkers to predict their response and assess survival.

Objectives: We aimed to evaluate the ability and clinical significance of the immune biomarker sIL-2R in HNC patients, to assess therapy response and prognosis.

Methods: We evaluated 328 blood samples from 145 Head and Neck Cancer patients (HNC) from several subgroups: 84 Larynx Carcinoma pre and 39-post therapy, 46 Oral Cavity pre and 29-post therapy, 12 Nasopharynx, 16 Parotid and other salivary gland patients. The control group included 45 healthy subjects. Serum sIL-2R levels were evaluated by ELISA assays and correlated to disease grade, lymph nodes, response to therapy, survival and cancer differentiation.

Result: Significantly higher sIL-2R levels were recorded in all HNC patients, as opposed to controls, in advanced vs low grade disease-which decreased following therapy. sIL-2R distinguished best, in comparison to other Tumor Markers, between HNC patients and controls. Survival was strongly associated to lower sIL-2R levels in patients entering the study.

Conclusions: sIL-2R is a sensitive immune marker for HNC patients. Its levels correlate to disease grade, assess response to therapy and are predictive of recurrence or a better survival. We suggest therefore using sIL-2R as a reliable prognostic marker in HNC patients as single marker, or in a combined panel of biomarkers.

Funding Source: Laboratory Fund

Serum HER-2 as A Sensitive Tumor Marker in Breast Cancer Patients

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Hypothesis: The HER-2/ neu oncogene is overexpressed in Breast Cancer (BC). The serum HER-2 assay was cleared by the FDA in 2000 for monitoring Metastatic Breast Cancer patients (MBC). Its levels were found elevated in about 25% of primary BC and in 25-75% of MBC patients.

Objectives: To evaluate the sensitivity of the serum HER -2 Tumor Marker in Israeli BC patients, as to treatments response evaluation (hormonal or targeted therapy), prediction of recurrence or metastases and prognosis of those patients.

Methods: 245 BC patients and 100 normal controls were evaluated for serum HER-2 levels, compared to established Tumor Markers - CA 15-3, CA 125, CEA, also the cytokeratin TPS, and their levels were correlated to disease activity, metastases- number and their location, response to treatment, prognosis and survival.

Result: Significantly higher levels of HER-2 (and also of the other Tumor Markers) were recorded in MBC- 31.7+4.6, as opposed to No Evidence of Disease patients- NED -12.4+0.46 and Controls- 11.9+0.24, which were very similar. Increasing levels of HER-2 recorded during patients monitoring (6.2 up to 38,15), were correlated to recurrence or Metastasis formation, shown only later (3-8m) by CTs or MRIs. Decreases in HER-2 levels (39.46 to 12.78) were correlated to treatments response, both hormonal and targeted therapies, to a better clinical outcome and survival.

Conclusions: Serum HER-2 is a sensitive Tumor Marker for BC patients, indicating response to treatments and detecting earlier recurrence or metastases formation than CTs or MRIs- a lead time which could enable treatments initiation or changes and might predict which patients will benefit from new treatments.

Funding Source: Laboratory Fund

Phenothiazines Modulate Chromatin-Related DNA Repair Signaling in Tumors

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Hypothesis: Tumor cells with deregulated epigenetic signaling may be responsive to phenothiazine (pheno) treatment due to increased phosphorylation of DNA damage response proteins.

Objectives: Epigenetic alterations have been recognized as factors in malignant transformation. Attacking these alterations pharmacologically has recently emerged as a promising strategy to combat tumors, as exemplified by histone deacetylase inhibitors (HDACi) in acute myeloid leukemia (AML). We have shown that the pheno family of neuroleptics, in combination with DNA damaging agents can cause hyperactivation of two important DNA damage response (DDR) factors, DNA-PKcs and ATM. In this study we aimed to understand if pheno can be used to treat AML and other tumors where alteration in the epigenome is one of the malignant drivers.

Methods: Connectivity Map (cmap) was applied on public domain gene expression data on tumor/normal cells exposed to pheno to reveal putative action mechanisms. Trypan blue exclusion and MTT cell viability assay were used for profiling cytoxicity in AML, small cell lung cancer (SCLC) and neuroblastoma (NB) cells. Effect on DNA-damage and apoptotic cell signaling was analyzed by western blot, flow cytometry, proximity ligation in situ and immunoprecipitation.

Result: In silico analysis of pheno-induced gene expression signatures revealed a profile similar to the HDACi trichostatin A and vorinostat, suggesting a role of pheno to chromatin-related processes. Pheno blocked AML, NB and SCLC growth alone or combined with (i) pan-HDACi panobinostat, (ii) BRD4 antagonist JQ1 or (iii) chemotherapeutics daunorubicin or gemtuzumab ozogamicin (GO). A high basal level of phosphorylation of the DDR proteins, DNA-PK and RPA32 as well as of the DNA DSB marker γH2AX and the epigenetic signalling component histone H3K18 were evident. Moreover, pheno also impaired repair of endogenous DNA DSB in AML cells and increased DNA-PKcs autophosphorylation and caused binding of DNA-PKcs to H3K18. A similar response was evident after combined pheno and HDACi/BRD4i in NB and SCLC cells which also resulted in both increased apoptotic and autophagic cell death.

Conclusions: Our results suggest that pheno may offer a novel therapeutic avenue either alone or in combination with agents targeting the epigenome in AML and other tumor malignancies.

Funding Source: The Cancer Society in Stockholm, Swedish Cancer Society

Evaluation of an Improved Version of the Arocell TK 210 ELISA for Determining TK1 Protein Levels In Serum Samples

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Hypothesis: Thymidine kinase 1 (TK1) is an ATP dependent enzyme involved in DNA precursor synthesis and it leaks into blood in patients with different malignancies where it forms stable aggregates. Serum TK1 activity measurements have been used in tumor markers diagnostics for many years. AroCell AB has recently developed an ELISA for TK1 protein determination, which can detect inactive forms of TK1, often found in patients with solid tumor diseases. However, the use of a serum matrix in the calibrators of the assay has significantly reduced the capacity to detect TK1 in sera from healthy persons. Our hypothesis is that an altered blank procedure can improve the detection range of the TK1 ELISA in sera with low TK1 protein levels.

Objectives: The main objective of the study is to improve the measuring range of TK 210 ELISA by shifting the blank from low TK1 serum matrix (without added recombinant TK1) to a sample dilution buffer.

Methods: AroCell TK 210 ELISA kits were used in this study. The sandwich ELISA is based on two monoclonal antibodies against the C-terminal region of TK1. In this assay, pre-incubation of sera with sample dilution buffer (SDB) is needed and this follows the routine ELISA procedure. Different concentrations of recombinant TK1 in selected pool of low TK1 sera, are used as calibrators in the standard protocol. TK1 protein levels in 271 healthy persons [148 women and 123 men, between 25 to 67 years of age] were determined by using the AroCell TK 210 ELISA. TK1 protein levels were calculated by using the low serum matrix blank and the SDB blank.

Result: Use of SDB as a blank instead of the low serum matrix gave a significant difference in the mean TK1 protein levels. The TK1 protein concentrations were in the range of 0 to 2.19 ng/mL (mean+/SD=0.08+/0.16; median =0.02) by using the standard protocol.
However, the SDB blank changed the detection range from 0.05 to 2.20 ng/mL, giving a mean+/SD = 0.25+/0.15; median = 0.24 ng/mL. There was no significant difference between men and women in TK1 protein levels calculated by either of the methods in this case.

Conclusions: This improved version of the AroCell TK 210 ELISA broadens the detection range of TK1 protein levels so that TK1 protein could be detected in sera from more than 95% of healthy persons compared to approximately 50% when using the earlier standard protocol.

Funding Source: This study was supported by AroCell AB.

Sensitivity of Neuroblastoma Cell Lines to Retinoids in Relationship to The Expression of Selected Protein Markers

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Hypothesis: Neuroblastoma (NBL) is the most common extracranial solid tumor in children, accounting for 6-8% of all childhood cancers. NBL is a complex and heterogeneous disease with several factors determining the clinical outcome, especially the age at diagnosis, stage of the disease as well as biological features of the tumor. Administration of retinoids represents important part of high-risk NBL treatment as it could delay or prevent tumor relapse after myeloablative therapy. Nevertheless, about 50% of these patients were resistant to this treatment or developed resistance to retinoids during therapy. Several putative biomarkers indicating sensitivity or resistance to retinoids were reported in recent publications focused on understanding to the mechanism of resistance to retinoids.

Objectives: The main aim of our study was to analyze the expression of selected candidate proteins (PBX1, HOXC9, HMGA1, HMGA2 and DDX39A) in relation to the sensitivity or resistance to retinoids together in a panel of 19 NBL cell lines.

Methods: In this study, 19 patient-derived NBL cell lines were used for experiments. Sensitivity or resistance to natural (all-trans retinoic acid, 13-cis retinoic acid, 9-cis retinoic acid) and synthetic (fenretinide, bexarotene) retinoids was determined by MTT assay. Endogenous expression of the candidate biomarkers were analyzed both on mRNA (RT-PCR) and protein (immunoblotting) levels.

Result: Obtained results showed increased expression of HMGA1, HMGA2 and PBX1 in resistant NBL cell lines. Furthermore, we observed reduction of viability after treatment with synthetic retinoids in these cell lines, which seemed to be resistant to the treatment with natural retinoids.

Conclusions: Our experimental study on the panel of patient-derived cell lines confirmed the association of selected putative markers with sensitivity or resistance of NBL cells to retinoids. In the next step, these results will be compared with expression levels of these markers in original tumor tissues, from which the cell lines were derived.

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microRNAs as Molecular Markers in Central Nervous System Lymphomas

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Hypothesis: Differentiation of central nervous system (CNS) lesions remains challenging, in spite of numerous diagnostic possibilities, including imaging techniques, cytological and flow cytometry examination of cerebrospinal fluid (CSF), and histological examination of stereotactic biopsy material. microRNAs might serve as new tools for earlier, faster and more precise differential diagnosis of primary lymphomas and nonmalignant lesions in the CNS.

Objectives: We aimed to assess the diagnostic value of miR-21, miR-19b-1 and miR-92a-2, miR-155, miR-196b, miR-let-7b, miR-125b and miR-9 expression in CSF and brain biopsies (BB) from patients with primary CNS lymphomas (PCNSL) vs. neurological CNS lesions.

Methods: microRNAs were assessed by RT-qPCR, with miR-24 as a reference, in CSF leftover samples and formalin-fixed paraffin-embedded samples from stereotactic BB collected for the routine diagnostic purposes from patients suspected of brain lymphomas (n=26) or neurological CNS lesions (n=59), consulted at the M. Sklodowska-Curie Memorial Cancer Centre and Institute of Oncology in Warsaw.

Result: 1. In the CSF, there were significantly higher levels of miR-21, miR-19b-1 and miR-92a-2 in patients with PCNSL than in patients with nonmalignant neurological lesions. CSF levels of the three miRNAs differentiated PCNSL from neurological lesions, with 54% sensitivity and 90% specificity.
2. In BB samples, the expression of miR-21, miR-19b-1 and miR-92a-2 did not differ between lymphomas and nonmalignant lesions.
3. In BB samples, miR-155 and miR-196b were significantly overexpressed and miR-let-7b, miR-125b and miR-9 were downregulated in PCNSL vs. nonmalignant neurological diseases.

Conclusions: miRs emerge as promising diagnostic markers that may support earlier PCNSL treatment decisions, thus improving patient outcome. Further developments will include validation of miRs as markers on independent series of patients and NGS profiling of paired samples (CSF/brain biopsy) from PCNSL patients.

Funding Source: N/A

Deletion of DNA-Binding Inhibitor Id3 Enhances Anticancer Function of T Cells by Inducing Th9 Cells

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Hypothesis: Deletion of DNA-binding inhibitor Id3 promotes Th9 cell differentiation, so deletion of Id3 can enhance anticancer function of T cells by inducing Th9 cells.

Objectives: Th9 cells, a recently characterized subset of CD4 helper T (Th) cells, produce the pleiotropic cytokine IL-9 that has been suggested to be involved in anti-tumor immunity. The molecular mechanisms by which transforming growth factor-β (TGF-β) and interleukin 4 (IL-4) control the differentiation of Th9 cells remain incompletely understood. We found that TGF-β1 and IL-4 down-regulated the expression of DNA-binding inhibitor Id3, and this process required the kinase TAK1. The reduction of Id3 expression enhanced binding of the transcription factors E2A and GATA-3 to the Il9 promoter region and promoted Il9 gene transcription. Strikingly, even in the absence of exogenous IL-4, TGF-β1 was able to drive Id3-/- naïve CD4+ T cells to differentiate into Th9. As such, we want to investigate if Id3 could be a target of anticancer immunotherapy through Th9 cells.

Methods: A T cell adoptive-transfer system was used to treat tumor in an experimental melanoma mouse model. As Id3–/– naïve T cells could differentiate into Th9 cells in the cultures with TGF-β1 alone in the absence of exogenous IL-4, we cultured Id3−/− or wild-type naive CD4+CD25− T cells for 3 days with anti-CD3 and anti-CD28, together with TGF-β1, and injected the cells intravenously into Rag1−/− mice (which have a congenital deficiency in mature B cells and T cells); We then subcutaneously injected B16 melanoma cells into the Rag1−/− recipient mice. We also confirmed the instinct anti-tumor effect of Id3-deficient Th9 cells from Id3f/f Cd4-Cre+ mice that excluded the possibility of an effect of the systemic Id3 deficiency of Id3−/− mice on their thymic development.

Result: Mice that received Id3−/− T cells consistently showed slower tumor growth than that of mice that received wild-type T cells. Importantly, treatment with anti-IL-9 antibody significantly abolished the inhibition of melanoma growth in mice that received Id3−/− CD4+ T cells. However, treatment with neutralizing antibody to IL-9 had no effect on tumor growth in tumor-bearing mice that received wild-type CD4+ T cells. Adoptive transfer of TGF-β1-treated naive CD4+CD25− T cells from Id3f/f CD4-Cre+ mice into Rag1−/− mice, followed by challenge of the recipient mice with melanoma cancer cells also suppressed the growth of the tumor. Again, the administration of anti-IL-9 antibody completely abolished the anti-tumor effect of Id3f/f CD4-Cre+ T cells.

Conclusions: Our study reveals a crucial role for Th9 cells differentiated from Id3-deficient CD4+ T cells in the suppression of melanoma growth in mice, which might have implications in melanoma patients.

Funding Source: This research was supported by the Intramural Research Program of the NIH, NIDCR.

The Influence of PSA Autoantibodies in Prostate Cancer

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Hypothesis: The U.S. Preventive Services Task Force (USPSTF) has recommended against PSA-based screening for prostate cancer due to potential possibilities of false-results. Since no alternative test is available to replace it, we have initiated a trial with the hypothesis whether Galectin-3 (Gal-3) serum level and/or the patients' immune response to PSA and Gal-3 antigens could complement the PSA test as diagnostic tools for prostate cancer patients.

Objectives: The purposes of this study were 1- to determine the expression levels of AAPSA, Gal-3, and AAGal-3 as diagnostic accompaniments of the PSA test, and 2- to examine the relationship between PSA and AAPSA and between Gal-3 and AAGal-3 along with the clinical status of the patients enrolled.

Methods: A blinded, prospective, single institution, pilot study was conducted. A total of 95 men were recruited and classified into 5 different groups: healthy controls (Group1), newly diagnosed (Group2), no recurrence (Group3), rising PSA after local therapy (Group4), and metastatic patients (Group5). The primary endpoints were the levels of serum PSA, PSA autoantibodies (AAPSA), Gal-3, and Gal-3 autoantibodies (AAGal-3). Data were analyzed by Spearman's rank correlation (rho) and least squares linear regression modeling.

Result: The expression levels of PSA, AAPSA, Gal-3, and AAGal-3 were determined in both healthy controls and prostate cancer patients. Negative correlations were observed between PSA and AAPSA levels among all 95 men combined (rho = -0.321, P = 0.0021; fitted slope -0.288, P = 0.0048), and in metastatic patients (rho = -0.472, P = 0.0413; fitted slope -1.145, P = 0.0061), while AAGal-3 was not significantly associated with Gal-3 level.

Conclusions: We suggest an association between PSA and AAPSA, whereby the AAPSA may alter PSA levels. It provides a novel outlook for prostate cancer diagnosis, and should serve as a basis for an all-inclusive diagnostic trial centering on patients with metastasis.

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Transcriptomic and Methylome Approaches to Identify Genetic Alterations in Esophageal Cancer of India

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Hypothesis: Fermented Betel Quid may play a major role in progression of Esophageal Squamous cell Carcinoma (ESCC) of High-Risk -region of India through genetic and epigenetic alterations.

Objectives: 1. To identify Chromosomal Level Changes in ESCC patients associated with Fermented Betel quid.
2. To Identify transcriptomic level changes in ESCC patients associated with Fermented Betal Quid.
3. To Identify gene specific promoter methylation in ESCC patients associated with Fermented Betel Quid.

Methods: Genome-wide analysis for chromosomal alterations by GeneChip® Human Mapping 10K Array Xba 142 2.0, gene expression profile by cDNA microarray and Infinium 450k Array (Illumina) for genome-wide methylation profiling were performed in esophageal cancer from a high-incidence region of India, where tobacco use and alcohol consumption are widespread and the users of which are also betel quid chewers. To understand the role of chemical compounds present in betel quid associated with the progression of ESCC, genes located at amplified region and deleted region, genes with differential expression found in this study, and genes that were associated with hyper-methylation and hypo-methylation were analyzed for their association with chemical compounds found in tobacco smoke (arsenic, benzene, cadmium, formaldehyde, and NNK) and betel quid (arecoline and aflatoxin B1) using Comparative Toxogenomic Database.

Result: Most of the genetic alterations in our study were mostly associated with aflatoxin B1 which is produced by fermented areca nut, a main component of betel quid, as compared to NNK and arecoline. Up-regulated/Amplified and promoter hypomethylation of genes related to aflatoxin B1 such as FGF12, FLNC, GPR87, OSMR, RHOJ, THEM2, CD14, ARG1, SLFN5, SPA17, CD44, ENAH, INSIG2, PLK2, S100A3, TWIST1, TRIM29, FBN2, UGT2B4, NPC1, and TAC3 are involved in Cell Proliferation, Invasion and Migration. Down-regulated/ Deleted and Promoter hypermethylated of tumor suppressor or metastasis suppressor or cell adhesion or apoptotic inducing genes such as LZTS1, COL12A1, MTUS1, GPR56, DIAPH3, CD9, CSTA, CSTB, TLN2, EPHX1, COL4A4, PF4 & GLIPR1, RND1, RND3, are associated with aflatoxin B1.

Conclusions: Our findings provide a new basis for improving the understanding of aflatoxin B1 induced effects in contribution to development of ESCC. These candidate genes would serve as novel genetic markers to delineate molecular pathogenesis of betel-related esophageal lesions in high-risk area of India.

Funding Source: N/A

Investigation of Putative Molecular Marker in Human Papillomavirus Associated Cervical Cancer in Indian Population

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Hypothesis: Cancer of uterine cervix, the most common cancer in Indian women caused due to persistent infection with high-risk Human papillomaviruses (HPVs) types particularly type 16/18. However, HPV infection alone is not sufficient, but in conjunction with host genetic factors which play a plausible role for the development of cervical carcinogenesis

Objectives: The high prevalence, death and economical burden put cervical cancer on higher priority for scientific investigation in India. Prevalence of allelic variations related to cervical cancer has been found to differ across different population. Studies have established that Indian population is genetically different in terms of allelic distribution for disease causing variations. There is no large scale study till date investigating role of genetic variations toward cervical cancer pathophysiology. Hence, in this study we have investigated cervical cancer susceptibility in Indian women.

Methods: Here in this study 32 single-nucleotide polymorphisms (SNPs) were selected from various genes having role in apoptosis, cell differentiation and cell signaling in 716 subjects consisting of 469 cases (142 pre-cancer + 327 invasive carcinoma) along with 247 healthy controls, a case-control based association study through Sequenom MassARRAY platform. In addition, protein expression of genes whose genotypic frequency was found to be significantly associated was also evaluated by protein expression.

Result: Current study evaluated association of host genetic factors in the progression and manifestation of cervical cancer in Indian population. The association analysis for combined samples (cancerous and precancerous together) verses control showed association of variants with eight SNPs in MMP-9, P73, MDM2, MYC, ETS-1, c-JUN and MMP-7 at significance level.

Conclusions: The MMP-9, p73, MDM2 and ETS1 could have a predisposition or may exhibit susceptibility for cancer/precancer development or HPV infection, whereas the SNP in c-Jun and MYC may exhibit strongly protective against the risk of HPV infection/cervical cancer. The studied variants may contribute toward defining better prognostic or diagnostic markers for HPV-mediated cervical cancer in northern Indian population.

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Investigation of Snps/Haplotypes as Biomarkers to Tobacco Associated Oral Squamous Cell Carcinoma in Indian Population

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Hypothesis: Oral cancer is one of the most common cancer in the Indian population having strong interaction of environmental (smoking, tobacco and alcohol consumption) and genetic factors. Despite the risk of nicotine exposure, some of the patients do not develop oral cancer, therefore, there must be other factors which influence tobacco-exposed individuals in the succession of a malignancy and may include a combination of total tobacco exposure and genetic susceptibility.

Objectives: The high prevalence, death and tobacco use put oral cancer is one of the major cancer of investigation in India. Prevalence of allelic variations in immunomodulatory genes related to oral cancer has been found to differ across different geographic location. Studies have established that Indian population is genetically different in terms of allelic distribution for disease causing variations. There is no large scale study till date investigating role of genetic variations with life style toward oral cancer in India. Hence, in this study we have investigated SNPs/Haplotypes of genes of Th1/Th2 pathway in oral cancer susceptibility in Indians.

Methods: Here in this study 22 single-nucleotide polymorphisms (SNPs) were selected from various genes of Th1/Th2 pathways in 400 subjects consisting of cases (50 pre-cancer + 100 invasive carcinoma) along with controls, a case-control based association study using high throughput denaturing high-performance (dHPLC) chromatography followed by sequencing.

Result: Identified three novel variations in IL-10 (NCBI Gene Bank accession number KT153594, KT291742 and KT291743) in this population in addition of other reported SNPS. In addition, in TNF-LTA locus, two haplotypes ATCTGG and ACACGG, were identified as risk haplotypes for oral cancer risk and also positively associated with life style habits [tobacco chewing (P=0.04, OR=3.4) & socio-economic status (P=0.01, OR= 3.4)]. The percentage of risk haplotypes was found 3 fold higher in precancer while 4 fold in advance stages of oral cancer as compared to controls.

Conclusions: The study showed that the identified novel SNPs or the haplotypes in this population may be suitable candidate for future biomarker which need more detail study in Indian population.

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Soluble Epha2 Has a Potential of Novel Serum Biomarker for Malignant Cancers

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Hypothesis: EphA2 is a tyrosine kinase receptor that is highly expressed in many aggressive human cancers including lung, ovarian and pancreas cancers. Soluble form of EphA2 (sEphA2) is also released from cancer cell surface and will be detected in serum specimens of various cancer patients.

Objectives: It is known that patients with a highly positive surface expression of EphA2 carcinomas have poor prognosis, therefore, preclinical and clinical studies using antibody targeting EphA2 are underlying. Recently we established a new mAb to EphA2 and developed sandwich ELISA system detecting human sEphA2 with high sensitivity and specificity. Measuring the serum concentration of sEphA2 in the serum specimens of healthy donors or malignant cancer, we examined the usefulness of sEphA2 as a novel biomarker for malignant cancers.

Methods: Using various cancer cell lines, we tested the expression and secretion of EphA2 by flow cytometry analysis and sandwich ELISA. We quantified serum sEphA2 levels in healthy donors (n=40), patients with lung cancer (n=15), pancreas cancer (n=13), ovarian cancer (n=26), and breast cancer (n=10). Serum levels of sEphA2 were also compared with other diagnostics biomarkers such as CEA, CYFRA and CA125.

Result: Human carcinoma cell lines including lung, pancreas and ovarian carcinoma expressed EphA2 highly, and released sEphA2 into the culture supernatant. The serum levels of patients with malignant cancer are significantly higher than those of healthy donors. There was no correlation between sEphA2 and other diagnostic markers except CYFRA.

Conclusions: We evaluated increased levels of sEphA2 in serum specimen of various cancer patients, indicating a new candidate for serum biomarker of cancer.

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Carboxypeptidase M (CPM) Contributes Tumor Growth and Metastasis Related in Epithelial Mesenchymal Transition in Human Esophogeal Carcinoma

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Hypothesis: Carboxypeptidase-M (CPM) is a membrane-bound exopeptidase that is able to process a multitude of different substrates, among them growth factor, cytokine and chemokine like EGF, SDF-1 and CCL1. CPM preferentially expressed on cancer cell surface has been suggested in the contribution of tumor growth and metastasis.

Objectives: The aim of this study is to examine the properties of CPM for tumor growth and metastasis in human carcinoma cells and to investigate as a candidate of biomarker in epithelial-to-mesenchymal transition (EMT).

Methods: We recently established the monoclonal antibody specific for human CPM and found the constitutive expression of CPM on various human cancer cells. Silencing CPM expression by small interfering RNA (siRNA) specific for CPM in esophageal and breast carcinoma cells were examined by flow cytometry and confocal laser microscopy. Cancer cell growth, motility and EMT-related gene expression were examined by alamar blue assay, scratch assay and RT-PCR.

Result: Treatment of cancer cells with EMT-mediated cytokines, including TGF-beta, IL-6 and OSM, increased CPM expression on their cell surface and enhanced cell migration. We found that CPM-silenced carcinoma exhibited reduced cell proliferation not only in 2D culture flask but also in 3D spheroid culture plate. We also found that CPM-silenced carcinoma exhibited reduced motility of cell in wound healing assay. Furthermore, treatment of siRNA for CPM inhibited the expression of EMT-related genes such as snail and slug that are important for cancer motility.

Conclusions: Taken together, these data indicate that CPM contributes to cell growth and EMT and might be a diagnostics and/or therapeutic target in esophageal carcinoma.

Funding Source: Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan

Development and Evaluation of a Fully-Automated Chemiluminescent Immunoassay for PIVKA-II (ARCHITECT® PIVKA-II)

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Hypothesis: Using a new antibody for PIVKA-II, we can develop a fully-automated PIVKA-II assay which is less sensitive to blood collection tube type, shows improvement of the variation near the cut-off value and demonstrates excellent analytical performance for clinical use worldwide.

Objectives: PIVKA-II (protein induced by vitamin K absence or antagonist-II) is used as an aid in the diagnosis, monitoring and management of hepatocellular carcinoma. This maker is mainly used in Japan. Existing PVIKA-II assays show variability to blood collection tube types. We developed a new antibody and assay for PIVKA-II. We characterized the antibody and its epitopes, developed a new assay and evaluated the analytical performance of that assay (ARCHITECT PIVKA-II).

Methods: The epitope characterization was performed by using prothrombin Gamma-carboxyglutamic acid residues (Gla) domain polypeptides. Precision, sensitivity, linearity and potential interferences were performed based on guidance from each Clinical and Laboratory Standards Institute (CLSI) Document. Correlations to Picolumi and Lumipulse PIVKA-II assays and to serum and plasma were performed with samples sourced from Shinshu University Hospital. Tube type equivalency was evaluated.

Result: This new anti-PIVKA-II antibody (3C10) detected equivalent epitopes with the on-market PIVKA-II antibody (MU-3) and had equivalent reactivity to MU-3. The total imprecision showed %CVs of 3.2-3.9. The LoB, LoD and LoQ ranged from 0.45 to 0.64, from 1.05 to 1.45 and from 4.93 to 5.06 mAU/mL. The assay is linear up to 30,000.00 mAU/mL. There were no differences between sample types and no interference of common drugs and endogenous substances was observed. The correlation between the Picolumi PIVKA-II or Lumipulse PIVKA-II and the ARCHITECT PIVKA-II or between serum and plasma on ARCHITECT PIVKA-II were good. No difference between a plain serum tube and a variety of tubes was observed on ARCHITECT PIVKA-II.

Conclusions: We developed a PIVKA II assay which is less affected by blood collection tube type and has low variation near the cut-off value. The anti-PIVKA-II antibody 3C10 had equivalent epitopes and reactivity to PIVKA-II with MU-3. The ARCHITECT PIVKA-II assay demonstrated good analytical performance and compared well with other on-market assays.

Funding Source: N/A

Diagnostic Performance of Tumor Makers AFP and PIVKA-II in Chinese Hepatocellular Carcinoma Patients

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Hypothesis: Early detection of HCC plays an important role in effective control of hepatocellular carcinoma (HCC). Tumor makers are useful for early diagnosis and monitoring of recurrence of HCC. Although α-fetoprotein (AFP) has been widely used as an indicator of HCC, it has its limitations. Prothrombin induced by the absence of vitamin K or antagonist-II (PIVKA-II) is a sensitive diagnostic marker in HCC. The combined performance of AFP and PIVKA-II in early detection of HCC is not clear.

Objectives: The aims of this study were to determine (1) performance of PIVKA-II for the diagnosis of HCC (2) the combined performance of AFP and PIVKA-II (3) comparison between the ARCHITECT and FUJIREBIO platforms.

Methods: A total of 200 healthy subjects, 250 non-HCC liver patients and 148 HCC patients were enrolled in this study. Serum AFP and PIVKA-II were measured on both the ARCHITECH and FUJIREBIO platforms.

Result: The sensitivity (SE) and, specificity (SP) of ACHITECH PIVKA-II was 76% and 89%, with a positive predictive value (PPV) of 69% and negative predictive value (NPV) of 92%. The SE, SP of ARCHITECH AFP was 68% and 91%, with PPV of 72% and NPV of 90%. The SE, SP of combined ARCHITECH AFP and PIVKA-II was 85% and 82%, with PPV of 61% and NPV of 94%. The SE, SP of combined AFP and PIVKA-II in early stage HCC patients was 81% and 82%, with PPV of 43% and NPV of 96%. The table below shows the sensitivity, specificity, positive predictive value, and negative predictive value with different predictor variables.

Conclusions: The combined AFP and PIVKA-II panel is a more accurate marker for HCC presence than AFP or PIVKA-II alone, especially in stage 1&2 HCC patients.

Funding Source: Abbott Diagnostics

Epigenetic Profiling of Circulating Cell Free Nucleosomes – A Novel Approach to Reducing the Impact of Colorectal Cancer

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Hypothesis: Colorectal cancers develop via pre-cancerous adenomas and removal of high risk adenomas is impacting the CRC incidence rates in the US where screening colonoscopy is offered. However, limited compliance for colonoscopy and poor detection rates for Advanced Adenomas with alternate screening approaches, including stool testing and recently approved blood based testing approaches limit the potential for further reductions.

Circulating nucleosome DNA contains the same mutations as matched cancer tissue samples suggesting a tumor origin for at least some circulating nucleosomes. We have identified clinically distinct epigenetic profiles of nucleosomes in the blood of patients with colorectal cancers compared to healthy patients. Since genome wide epigenetic signals are altered early in tumorigenesis we hypothesized that epimutations may be present within precancerous lesions.

Objectives: Identification of diagnostically useful, differential nucleosome patterns in the blood of patients with Advanced Adenomas

Methods: We have developed ELISA tests for specific epigenetic features of circulating nucleosomes (NuQ®) including histone modifications and variants, DNA modifications and adducts between nucleosomes and non-histone proteins and show that the profile of epigenetic features can be correlated with clinical disease. Serum samples from patients with Colorectal Disease symptoms (10µl in duplicate) were analyzed using NuQ® assays employing coated capture antibodies against a conserved nucleosome epitope and various biotinylated profiling antibodies with streptavidin HRP/ABTS colorimetric readout. An algorithm for optimal diagnostic performance was developed using a linear regression.

Result: We present Nucleosomics® data from 530 symptomatic individuals including 246 subjects with colorectal adenomas, 49 subjects with stage I cancer and 48 subjects with stage II cancer. All the subjects had undergone colonoscopy. A panel of 5 NuQ® assays in an age adjusted linear regression model is able to detect 67% of the high risk adenomas at 78% specificity. Importantly, the same panel detected 81% of the early stage cancers.

Conclusions: Epigenetic features of circulating cf- nucleosomes are potentially useful biomarkers for the identifications of individuals with high risk Colorectal adenomas as candidates for early surgical or therapeutic intervention.

Funding Source: Volition is listed on the US NYSE Mkt Stock Exchange (VNRX)

A Novel Biomarker for Early Diagnosis of Hepatocellular Carcinoma

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Hypothesis: Monomeric laminin γ2 (Ln-γ2) is frequently expressed in malignant tumors, and its expression is associated with disease progression and metastasis of cancer cells including hepatocellular carcinoma (HCC) cells. Hence we hypothesized monomeric Ln-γ2 has the possibility to be a biomarker in HCC diagnosis.

Objectives: Ln-332, a heterotrimer composed of α3, β3, and γ2 laminin chains, is a major component of the basement membranes. In contrast, Ln-γ2 is frequently expressed as a monomer form in malignant tumors, and is believed to be a specific biomarker of malignant tumors. However, there is no direct way to detect monomeric Ln-γ2, selectively, in the presence of Ln-332, because all available antibodies recognize both monomeric and heterotrimeric forms of Ln-γ2. Hence, we established a monoclonal antibody that reacts specifically with monomeric Ln-γ2 and developed a monomeric Ln-γ2 detection system, based on chemiluminescent immunoassay (CLIA). Although Ln-332 is not detected in normal liver, monomeric Ln-γ2 is frequently expressed in hepatocellular carcinoma (HCC) and plays crucial roles in the progression. The aim of this study is to examine whether serum monomeric Ln-γ2 is a biomarker for HCC diagnosis.

Methods: We measured serum levels of monomeric Ln-γ2 in healthy volunteers and in patients with HCC and chronic liver diseases (CLD) by CLIA using the specific antibody.

Result: Serum level of monomeric Ln-γ2 was significantly higher in patients with HCC compared to patients with CDL or healthy volunteers. Elevated monomeric Ln-γ2 levels were observed in 0% of healthy volunteers, 17% of patients with CLD, and 63% of patients with HCC. The positivity rate for the combination of monomeric Ln-γ2 and PIVKA-II in patients with HCC was 89.5%, which was better than that for either of the two markers alone (63% and 68%, respectively). Among patients with early stage HCC (T1 or T2), the positivity rates for monomeric Ln-γ2, AFP, and PIVKA-II were 61%, 39%, and 57%, respectively.

Conclusions: These results suggest that serum monomeric Ln-γ2 may be a potent biomarker for HCC surveillance. Furthermore, the combination of monomeric Ln-γ2 and PIVKA-II may be more sensitive for laboratory diagnosis of HCC than the combination of AFP and PIVKA-II.

Funding Source: Adaptable and Seamless Technology Transfer Program through Target-Driven Research and Development (A-STEP) from Japan Agency for Medical Research and Development (AMED)

Abbott ARCHITECT® AFP and PIVKA II for Early Detection of Hepatocellular Carcinoma

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Hypothesis: Biomarker panels may aid in early detection of hepatocellular carcinoma (HCC) over the current practice.

Objectives: The Abbott ARCHITECT® AFP and protein induced by the absence of vitamin K or antagonist-II (PIVKA II) assays in combination may provide improved detection of early stage HCC. The study objective was to evaluate the performance of AFP and PIVKA II to aid in early HCC detection.

Methods: Retrospective case-control specimens collected at the Johns Hopkins Medical Institutions were used for this study, except the fibrosis specimens (University of Texas Southwestern Medical Center). Patient serum samples included chronic hepatitis (n=102), fibrosis (n=19), cirrhosis (n=40), hepatitis with cirrhosis (n=54) and HCC (n=69 stages 1 & 2, n=49 stages 3 & 4), and normal individuals (n=34). Random Forest algorithm was used to develop the predictive models, and included AFP and PIVKA II alone and in combination with Age and Gender as predictor variables.

Result: Training samples for the Random Forest predictive models included 69 stage 1 & 2 HCC (positive) patients and 215 non cancers without normal subjects. The table shows performance of the trained models with different predictor variables with a cutoff of p=0.5.

Model	Predictor Variables	AUC	Sensitivity	Specificity
1	AFP	0.88	0.49	0.95
2	PIVKA II	0.87	0.35	0.95
3	Age, Gender, AFP	0.93	0.67	0.94
4	Age, Gender, PIVKA II	0.91	0.52	0.94
5	Age, Gender, AFP, PIVKA II	0.95	0.71	0.97

The area under the curve (AUC) for the best model utilizing age + gender + AFP + PIVKA II as predictor variables was 0.95 with a sensitivity of 0.71 and specificity of 0.97. The Random Forest model using the same set of predictor variables have also been trained for a sample with non-cancer without normal (n=215) vs. all HCC (n=118), and the AUC for this trained model was 0.97 with a sensitivity of 0.82 and specificity of 0.95.

Conclusions: The model age + gender + AFP + PIVKA II showed superior sensitivity and specificity for HCC detection compared with AFP and PIVKA II alone or individually combined with age and gender. Follow-up studies are recommended to validate the algorithm for the proposed clinical utilities.

Funding Source: Abbott Diagnostics

Improvement in Performance of the EarlyCDT-Lung Test for Detection of Lung Cancer by Addition of Antigenic Biomarkers

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Hypothesis: Antigenic biomarkers used in combination with autoantibody panels leads to improvement in assay the performance parameters of the combined assay compared with either types of biomarker alone.

Objectives: The EarlyCDT-Lung Test measures a panel of seven autoantibodies and is intended to be used as an aid to early detection of lung cancer. It has high specificity but moderate sensitivity. The aim of this study was to investigate the performance of known antigenic biomarkers in lung cancer both alone and in combination with EarlyCDT-Lung.

Methods: A cohort consisting of 97 cases of lung cancer (71% stage 1 and 2, 71% male, median age 61y) and 97 controls were selected from the Oncimmune biobank. All lung cancer cases were individually matched to controls by age, gender and smoking history. The antigenic biomarkers, CEA and CYFRA 21-1, were measured by ELISA (Fujirebio) according to manufacturers' instructions. The EarlyCDT-Lung test was measured according to Oncimmune standard procedures. Commercial cut-offs were applied for the EarlyCDT-Lung test and cut-offs for the antigenic assays were set manually to achieve high specificity.

Result: The performance of each of the biomarkers alone and in combination is given in Table 1

Biomarker	Sensitivity	Specificity	PPV*
EarlyCDT-Lung	25.0%	93.4%	8.5%
CYFRA 21-1	27.1%	97.8%	11.1%
CEA	17.7%	98.8%	11.2%
EarlyCDT-Lung plus CYFRA 21-1	44.8%	91.2%	11.1%
EarlyCDT-Lung plus CEA	39.6%	92.3%	11.2%
EarlyCDT-Lung plus both antigens	54.8%	91.2%	13.3%

PPV (Positive Predictive Value) assumes 2.4% lung cancer prevalence

Conclusions: Combining antigenic markers with autoantibody panels leads to increased sensitivity whilst maintaining high specificity. The improved assay performance characteristics could lead to greater clinical utility. The antigens were measured using ELISA in this study and so will be easily integrated into the EarlyCDT-Lung platform.

Funding Source: Oncimmune Ltd

Targeted Exome Sequencing of Cancer-Related Genes in Human Cancers Using the Semiconductor Sequencer

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Hypothesis: Targeted exome sequencing using the semiconductor sequencer will be a sensitive and effective approach for detecting multiple genetic alterations (single nucleotide variants, insertions/deletions, and copy number variations) in human cancers.

Objectives: Next-generation sequencing technologies have revolutionized cancer genomics research by providing a comprehensive method of detecting somatic cancer genome alterations. Platforms for genomic DNA alterations are more common in clinical practice and include whole genome/exome sequencing analysis. These tests are still very expensive, although the costs are coming down substantially. Here, we aimed to determine the efficacy and advantages of targeted exome sequencing of known cancer-related genes in human cancers.

Methods: DNA was extracted from 19 human cancer cell lines and 62 human cancer specimens and their corresponding non-cancerous tissues, including oral squamous cell carcinomas (OSCCs) and multiple myelomas (MMs). Forty nanograms of DNA were used for multiplex PCR amplification with an Ion Ampliseq Comprehensive Cancer Panel that offers targeted coverage of all exons in 409 tumor suppressor genes and oncogenes. This platform was designed to be amplification based capture with 15,992 regions. Purified DNA libraries were sequenced with 6-8 samples on Ion Proton P1 chip.

Result: Each sample underwent on mean 8.4 million sequencing reads after quality filtering. The mean base coverage depth was 530, and >95% of targeted bases were represented by at least 20 reads. The number of non-synonymous somatic mutations in 46 patients with OSCC ranged from 1 to 21 with a mean of 7.5. The most frequent mutations in OSCC were in TP53 (63.0%), NOTCH1 (21.7%), CDKN2A (17.4%), SYNE1 (15.2%) and TAF1L (15.2%). We also detected a mean of 6.1 (range 3-11) non-synonymous mutations per MM patient. Somatic mutations were found in known MM-associated genes, including TP53 and NRAS. Pathway assessment has shown that somatic aberrations within MM genomes are mainly involved in several important pathways, including cell cycle regulation, RTK–MAPK-PI3K and NF-kB.

Conclusions: This study demonstrates the utility of using a semiconductor-based sequencing to efficiently identify human cancer mutations. The targeted next-generation sequencing using low amounts of FFPE DNA is a valuable tool for rapid (2 days) and high-throughput genetic testing in research and clinical settings.

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Prostaglandin E2 Is an Important Biomarker in Prostate Diseases and In Andrology

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Hypothesis: The death rate from cancer is one of the first places in all the world, and among males, prostate cancer has a high percentage. On the other hand, diseases of the prostate are found in almost all age groups and fertility problems created. The leading place belongs to benign prostatic hyperplasia (BPH), prostate cancer (PCa) and unspecific chronic prostatitis (ChP). According to some scientists, many cancer cells produce prostaglandin E2 (PgE2), which supports chronic inflammation to form a tumor microenvironment and suppresses the immune system.

Objectives: To study the content of PgE2 in blood sera and prostate secretions in patient with benign prostate hypertrophy, prostate cancer, chronic prostatitis and male infertility in different periods of the treatment.

Methods: PgE2 (R&D SYSTEMS) was detected by immunoassay in 230 samples of bodily fluids: 113 blood serum and 93 prostate secretion of patient, also 24 samples of donor blood serum.

Result: It was found that the PgE2 concentration in the blood has a deviation from the values in group of healthy subjects for all diseases of the prostate. Most interesting was the fact reduce PgE2 concentration in the blood of patients with BPH and prostate cancer compared to donors on average 2-3 (400-500 and 1100-1300 pg/ml, respectively). The level of PgE2 in prostate secretion increased to 50000 pg/ml, while for BPH only to 30000 pg/ml. Opposite changes PgE2 concentration in two biological fluids allow to calculate the coefficient for the clinical assessment of results. After complex treatment took place a positive change in the level of PgE2. The results are similar to data on ChPwere obtained for male infertility.

Conclusions: Especially in the synthesis of PgE2 diseased gland and its secretion in the bodily fluid can be used for differential diagnosis between PCa and BPH, ChP. The concentration of PgE2 in prostate secretion is higher than in the serum, which allows to conclude the possibility of using the test to a prostaglandin for non-invasive diagnosis of diseases of the male reproductive organs. The main obstacle to this instability in the laboratory reagents for immunoassay due to the high chemical reactivity PgE2.

Funding Source: Research in the laboratory of Astrakhan State Medical University

Differential Levels of Hypoxia-Inducible Factor-1a and 2a in Hepatocellular Carcinoma

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Hypothesis: Hypoxia-inducible factors (HIFs) are heterodimers which consist of hypoxia-regulated-alpha (HIF-a) and oxygen-insensitive beta subunit. Three types of α subunits have been found, HIF-1a, HIF-2a and HIF-3a have been identified. Though HIF-1a and HIF-2a are known to play an important role in carcinogenesis and cancer treatments, no study has been simultaneously examined HIF-1a and HIF-2a in hepatocellular carcinoma (HCC).

Objectives: The aim of this study was to examine both HIF-1a and HIF-2a in HCC.

Methods: HCC tissue samples from 206 patients and a number of HCC cell lines were used for this study. Immunohistochemistry and Western blot were employed to check the levels of HIF-1a and HIF-2a.

Result: We found that the level of HIF-2a in HCC tumor tissues was significantly lower than in peritumoral tissues, while the level of HIF-1a showed the opposite result, higher in tumor tissues than in paracancerous tissues. HCC cells with HIF-1a over-expression were more resistant to apoptosis than those without HIF-1a over-expression, whereas the inhibition of HIF-1a facilitated the apoptosis of HCC cells. In contrast to HIF-1a, the over-expression of HIF-2a induced apoptosis in HCC cells and the inhibition of HIF-2a expression achieved opposite results. In the cultured HCC cells, we found that both HIF-1a and HIF-2a proteins were not detectable in normoxia condition, but significantly increased in the acute hypoxia condition. During the prolonged hypoxia, HIF-1a protein was decreased though still higher than in the normoxia condition, however, HIF-2a protein remained at a high level.

Conclusions: The expression patterns of HIF-1a and HIF-2a are different in HCC, with the former being increased and the latter being decreased. HIF-1a appears to be anti-apoptotic while HIF-2a pro-apoptotic. The switch from hypoxia-inducible factor HIF-1a to HIF-2a-dependent transcription occurs during chronic hypoxia.

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Alternative Splicing of Estrogen Receptor Alpha in Hepatocellular Carcinoma

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Hypothesis: Wide type estrogen receptor alpha (ERa) is reduced but its splice variant (ERa36) is increased in hepatocellular carcinoma (HCC).

Objectives: The incidence of HCC is much higher in males than in females and the underlying mechanism is thought to be associated with female hormones. However, the role of ERa and ERa36 signaling in hepatocellular carcinoma (HCC) remain largely known.

Methods: We examined ERa and ERa36 in three cohorts, which included: (i) primary HCC patients (N = 76, cohort P), (ii) secondary HCC from metastatic colorectal cancer (mCRC) (N = 32, cohort S), and (iii) HCC from The Cancer Genome Atlas (TCGA) (N = 121).

Result: WtERa was downregulated and that ERa36 was upregulated in tumor tissues in both cohort P and TCGA data set. ERa36 was downregulated in tumor tissues in cohort S. In cohort P, wtERa was differentially expressed in gender ($P < 0.000$), age ($P = 0.004$), tumor number ($P = 0.043$), tumor size ($P = 0.002$), intrahepatic recurrence ($P = 0.054$). ERa36 was unequally expressed in different non-tumor liver status ($P = 0.040$). WtERa was negatively associated with overall survival (OS) and disease free survival (DFS) in cohort P. Compared with non-tumor tissues, the expression of ERa36 was increased in primary HCC but decreased in secondary HCC, showing opposite expression patterns of ERa36 between primary HCC and secondary HCC.

Conclusions: Primary HCC is associated with the decreased WtERa but increased ERa36. The expression pattern of ERa36 is different between primary HCC and secondary HCC, as the former with the increased ERa36 but the latter with the decreased ERa36. Therefore, the expression of ERa36 may be used to differentiate the primary HCC and the secondary one.

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TPS an Appropriate Marker for the Follow-Up Monitoring of Breast Cancer Patients after Radical Surgery

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Hypothesis: Multiplication of cytokeratins is one of the signs of cell division. During the progression of malignant tumours we can observe a proteolytic cleavage of soluble cytokeratin fragments. These fragments are released into the blood where they can be detected using in vitro diagnostic tests.

Objectives: Breast cancer is the most common malignancy and the second cause of death by cancer in women. Serum tumour markers: carcinoembryonic antigen (CEA) and cancer antigen 15-3 (CA 15-3) are routinely used in diagnostics, treatment monitoring and follow-up of patients with breast cancer. The aim of this study was to evaluate the ability of tissue polypeptide specific antigen (TPS) to predict relapse in patients after radical resection of the tumour and compare it with the long term using markers: CEA and CA 15-3 as well as the evaluation of the optimal timing of their measurement.

Methods: The study included 472 patients who underwent radical surgery for breast cancer. The recurrence of disease (local recurrence or distant metastases) during the follow-up period was observed in 60 (13%) patients and 412 (87%) patients remain in relapse-free status. Serum levels of TPS, CEA and CA 15-3 were determined in both groups at following intervals: 1, 3 and 6 month after surgery. All cancer diagnoses were histologically verified. Summary of statistical findings for age and serum levels of tumor markers is presented. Receiver operating characteristic (ROC) curves were plotted and area under the curve (AUC) calculated. The Wilcoxon test was used to compare values in both groups of patients.

Result: We founded statistically significant difference in TPS serum levels between relapsed and relapse-free group at first and sixth month after surgery. Median of TPS in relapsed and relapse-free group at first month was 75 IU/L and 45 IU/L respectively, $p=0.0339$ and at sixth month 83 IU/L and 47 IU/L respectively, $p<0.0001$. The results of ROC analysis showed AUC values: first month=0.60568, sixth month=0.71692.

Conclusions: TPS serum levels, which are determined in the first and sixth month after surgery, can be used as a predictive marker of relapse of breast cancer in patients undergoing radical surgery.

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The Cancer Cascade

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Hypothesis: Serine proteases are the mediating agent in several biological cascades including blood clotting, immune responses, and now the cancer cascade.

Objectives: In men with symptoms of prostate diseases the initiation of the cancer cascade is manifest by the expression of matriptase. This signals the beginning of the cascade followed by the kallikreins, MMP-7 and Hepsin. The expression of the serine proteases and the matrix metalloprotease 7 signal the presence of pre-malignant disease moving toward malignancy process. The presence of MMP-7 expression heralds the ultimate expression of Hepsin which is the agent for malignant transformation or metastasis.

Methods: All samples were assayed according to the Eliza protocol. Incubating 50µl of standard or sample in wells with immobilized first antibody and 50µl of biotin-labeled reporting antibody for 1HR at 30° C. Wells are then dispensed and washed 3x with wash buffer. Next, add 100µl streptavidin HRP and incubate 30 minutes at room temperature. Wells are then dispensed and washed 3x with wash buffer. Add 100µl of TMB substrate for 30 minutes at room temperature. Then add 100µl of stop reagent (2M H_2SO_4) in the wells and read the plate at 450 nm within 15 minutes.

Result: A total of 512 serum specimens have been collected from patients with suspected prostate cancer undergoing a biopsy procedure. 186 (36.3%) samples had no measurable expression of any of the six biomarkers, 103 (20.1%) had only 1 marker with measurable expression, 56 (10.9%) had 2 markers with measurable expression, 33 (6.4%) had 3 markers with measurable expression, 41 (8.0%) had 4 markers with measurable expression, 50 (9.8%) had 5 markers with measurable expression, and 43 (8.4%) had all 6 markers with measurable expression. Of the 103 specimens with only 1 marker expressed, almost half of the time (45.6%) Matriptase was the only marker expressed. Hepsin expression was only seen when at least 3 other markers were also expressed. Hepsin expression was also correlated with MMP-7 expression.

Conclusions: The advantage of the cancer cascade provides a road map to patients who are eminent for malignant transformation and there by provides an opportunity for the arrest of the cascade with inhibition of the serine proteases and ultimately arrest the progression towards metastatic disease including bone, lung, and liver disease.

Funding Source: Stage I Diagnostics, Inc

Developing Precision Medicine for Lung Cancer

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Hypothesis: Lung cancer is the biggest cancer killer of men and women in the USA and new approaches for diagnosis, prognosis, and treatment are urgently needed. This is especially true, since the vast majority of Americans have changed their life style habits and stopped smoking yet the impact of past smoking on lung cancer etiology will be with us for the next 50 years, and there are many cases of lung cancer arising in very light or never smokers. Thus, over 50% of newly diagnosed lung cancer patients are former smokers and 15% are life time never smokers (< 100 cigarettes lifetime).

Objectives: Lung cancers are divided into “non-small cell” (NSCLC, adenocarcinomas, squamous, carcinomas, large cell cancers and mixed types) representing 85% of cases and “small cell lung cancer” (SCLC, neuroendocrine lung cancers) representing the remaining 15%. NSCLCs are treated with various combinations of surgical resection, radiation, chemotherapy, targeted therapy and more recently immunotherapy.

Methods: SCLC are treated with combinations of radiation therapy and chemotherapy while targeted and immunotherapy are still undergoing clinical trials. There have been major advances in preoperative evaluation (to select the right patients for curative surgical and radiotherapeutic attempts) and the use of less invasive thoracic surgical techniques and technical details of thoracic radiation therapy (including stereotactic radiation therapy) which all help to improve patient outcomes.
The key is to develop and employ molecular biomarkers that help select the best systemic therapy for each individual patient. There have been major advances in lung cancer genomics, chemotherapy, targeted therapy, and immunotherapy that have brought dramatically improved quality and quantity of life to lung cancer patients including true long-term, disease free survival. However, the treatments are complex, costly, have significant side effects and various specific treatments work in only ~20% of patients

Result: Thus, CLIA certified tumor biomarker tests and “liquid biopsy” (blood biomarker tests of circulating tumor DNA for mutations) for tumor associated DNA mutations and mRNA, and immunohistochemical (IHC) expression changes are being developed, tested, and used in the clinic. Prominent examples include mutations testing to detect EGFR, KRAS, BRAF, EML4-ALK fusion mutations, and expression of immune checkpoint markers (such as PDL1, PD1) to help select targeted agents directed against these oncoproteins or the use of immune “checkpoint inhibitors” for individual patients. In addition, the preclinical and early clinical development of new targeted lung cancer therapy and immunotherapy approaches include the identification of tumor molecular “enrollment” biomarkers to allow selection of patients whose tumors exhibit characteristics that indicate they are likely responders to the individual therapies.

Conclusions: Such precision medicine requires coordination of obtaining tumor and blood samples by the patient care team, processing them by appropriate CLIA laboratory technology, and providing this information back to physicians and patients in a timely and understandable manner. In addition, the need to “rebiopsy” patients tumors (or monitoring of blood liquid biomarkers) if they develop resistance to the first line of treatment and subsequent molecular analyses to allow treatment with appropriate second and third line therapies are also required. Together, these approaches are revolutionizing our ability to care for lung cancer patients in a manner which dramatically improves the quality and quantity of their lives.

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Biopsy Levels of miR-375, miR-200b and miR-29c Associated with Prognosis of Head and Neck Cancers

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Hypothesis: Despite new approaches in treatment of head and neck squamous cell cancer (HNSCC), the overall 5-year survival rate for patients with HNSCC is only 50 % mostly because of the high rate of recurrences and advanced stage of disease by diagnosis. Thus, biomarkers with specific indications for diagnosis, prognosis, and prediction of therapeutic response for HNSCC are desperately needed.

Objective: Many studies proved that the aberrations in the MicroRNAs (miRNAs) expression are tightly connected with pathogenesis of human cancers, including HNSCC. In this study we focused on the evaluation of three miRNAs with supposed tumour-suppressive effect (miR-29c-3p, miR-200b-5p, and miR-375-3p) as diagnostic and prognostic marker of HNSCC.

Methods: miR-29c-3p, miR-200b-5p, and miR-375-3p were studied using quantitative real-time PCR expression levels in 42 tissue samples of HNSCC patients and histologically normal tumour-adjacent tissue samples of these patients. ANOVA followed by ROC analysis was performed.

Results: Primary HNSCC carcinoma tissues can be distinguished from histologically normal matched noncancerous tumour-adjacent tissues based on hsa-miR-375-3p expression (p=0.0001 and 2.63-fold lower in cancers, sensitivity 87.5 %, specificity 65 %). Additionally, significant decrease of hsa-miR-200b-5p expression was revealed in tumour-adjacent tissue samples of patients with node positivity (0.17-fold expression, 95% CI 0.03−0.87; p=0.035). Lower expression of hsa-miR-200b-5p and hsa-miR-29c-3p in HNSCC tumour tissue was associated with higher tumour grade. Consequently, survival analysis was performed. Survival analysis showed a significant effect of miR-29c-3p on overall survival in tumour-adjacent tissue (hazard ratio, HR = 0.27, 95% CI = 0.01 to 0.85). In addition, there was a significant effect of miR-29c-3p on recurrence-free survival in tumour tissues (HR = 0.31, 95% CI = 0.10 to 0.91).

Conclusions: hsa-miR-375-3p seem to be relatively promising diagnostic marker in HNSCC. This study highlighted the importance of histologically normal tumour-adjacent tissue in HNSCC progress (significant decrease of hsa-miR-200b-5p expression in tumour-adjacent tissue of patients with node positivity and low expression of hsa-miR-29c-3p in HNSCC tumour-adjacent tissue associated with worse prognosis).

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Tumor Cells Treated with KIOM-CRC#5 Induce a Tumor-Specific Immune Response

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Hypothesis: KIOM-CRC#5, an ethanol extract of a medicinal herb, works as an immunogenic cell death inducer that can reduce the cancer development and attenuate cancer growth in a mouse model of lung cancer.

Objectives: It is known that some chemotherapeutic agents and drug substances combined with anticancer drug not only kill tumor cells but also induce a tumor-specific immune response which causes dead cancer cells function as a vaccine that primes the immune system to attack other cancer cells. In this study, we predict that CRC#5 has the ability to elicit tumor-specific immune response result in the reduction and attenuation of cancer. To prove this, A549 human lung cancer cells were treated with KIOM-CRC#5 then the expressions and release of several molecular markers of immunologic cell death were measured. We also checked the induction of tumor-specific immune response of KIOM-CRC#5 using a mouse allograft model of Lewis lung carcinoma.

Methods: A549 cells were treated with various concentrations of KIOM-CRC#5 then the expressions and releases of calreticulin, HMGB1, and ATP were measured using ELISA and Western blot analyses. C57BL6 mice were injected with cell-death induced LL/2 (LLC1) mouse Lewis lung carcinoma cells, treated with either cisplatin only or in combination with CRC#5 and cisplatin, then re-challenged with viable LL/2 (LLC1) cells after 7 days of 1st injection. The incidence and growth of tumor were monitored for 4 weeks

Result: A549 cells treated with CRC#5 exhibited a typical immunogenic cell death, featuring calreticulin exposure, ATP release, and HMGB1 release. The tumor incidence of mice primed with CRC#5 and cisplatin co-treated LL/2 cells was lower than those of mice primed only with cisplatin treated LL/2 cells. The growth of tumor was also attenuated in the former group of mice.

Conclusions: These results show the elicitation of tumor-specific immune response by a medicinal herb extract in vitro and in vivo, suggesting that herbal medicines when combined with current chemotherapeutic agents, can be very useful anti-cancer agents with cancer-vaccine property.

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Changes of Serum Levels of New Biomarkers in Patient with Dutasteride Treatment (Pilot Study)

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Hypothesis: 5α-Reductase inhibitors are widely used in the treatment of benign prostatic hyperplasia. However, randomized clinical trials have raised concerns that their use may be associated with an increased risk of high-grade prostate cancer tumors that would ultimately lead to worse prostate cancer outcomes. A doubling factor is effective for maintaining the sensitivity and specificity of PSA for prostate cancer detection but to date, no study was addressed new biomarkers used in early cancer detection such as proPSA and Prostate Health Index (PHI).

Objectives: Monitoring changes in the serum levels of biomarkers PSA, freePSA, [-2] proPSA and PHI during dutasteride treatment after 3, 6 and 12 months in patient with benign prostate hypertrophy.

Methods: The Immunoanalytical Laboratory of the University Hospital in Pilsen examined sera of 30 patients from the Urology department of the University Hospital. We assessed the levels of PSA, freePSA, [-2]proPSA and we calculated the Prostate Health Index (PHI). Serum biomarkers were measured using the chemiluminescent DxI 800 instrument (Beckman Coulter, USA). SAS 9.3 software was used for statistical analysis.

Result: The mean levels of PSA decreased from 6.28 to 2.85 ug/L, [-2]proPSA from 14.1 to 3.0 pg/mL and PHI from 29.9 to 12.9 after 12 months. The median of decrease of PSA after 12 months was 0.5094 (95%CI 0.4180, 0.5941), [-2]proPSA 0.4795 (95%CI 0.3138, 0.5421) and PHI 0.5696 (95%CI 0.5000, 0.6622).

Conclusions: If the decision for a biopsy is only based on a increase in the serum PSA values, a variable percentage of potentially aggressive tumors cannot be diagnosed. The use of PHI may be helpful in this regard, but more data and verification in a larger cohort of cases is needed.

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Effect of an Ethanol Extract of D. sophia Seeds on Phase I and II Drug Metabolizing Enzymes and P-Glycoprotein Activity

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Hypothesis: The increasing practice of co-administering herbal medicines with conventionally prescribed cancer drugs has raised considerably the risk of potential drug-herb interactions. The goal of the present study is to elucidate the inhibitory effect of D. sophia seeds, one of the medicinal herb traditionally used for lung cancer on major human drug metabolizing enzymes and a drug transporter.

Objectives: Descurainia sophia seeds have a variety of pharmacological functions and been widely used in traditional folk medicine including symptoms typical for lung cancer. However, their effects on human drug metabolizing enzyme (DME) activities have not been elucidated. The present study investigated the inhibitory effects of an ethanol extract of D. sophia seeds (EEDS) on human Phase I/II (DMEs) and P-glycoprotein (p-gp) in vitro.

Methods: The enzyme activities of human Phase I (cytochrome P450s, CYPs), Phase II (uridine diphosphate glucuronosyltransferases, UGTs) DMEs, and the drug transporter P-gp were determined in the presence of various concentrations of EEDS using commercially available luminogenic assay systems. The mode of enzyme inhibition and the inhibitory constant (Ki) value of EEDS were graphically determined with Lineweaver-Burk double reciprocal plots and secondary plots, respectively.

Result: The enzyme activity assays showed that EEDS moderately inhibited the CYP1A2, CYP2C9, and CYP2C19 isoforms with half maximal inhibitory concentrations (IC50) of 47.3, 25.8, and 38.7 μg/mL, respectively. Graphical analyses with Lineweaver-Burk double reciprocal plots and secondary plots indicated that EEDS competitively inhibited CYP2C9 with a Ki value of 19.8 μg/mL; however, it inhibited CYP2C9 and CYP2C19 in a mixed mode with Ki values of 5.2, and 11.9 μg/mL, respectively. Other Phase I (CYP2C8, CYP2D6, and CYP3A4) and Phase II (UGT1A1 and UGT2B7) enzymes as well as P-gp were weakly or negligibly affected by EEDS with concentrations up to 500 μg/mL.

Conclusions: EEDS is a selective inhibitor of CYP1A2, CYP2C9, and CYP2C19 with moderate enzymatic inhibition. Clinically, full consideration should be given to a potential toxic adverse effect from a herb-drug interaction when drugs that are particularly susceptible to CYP1A2, CYP2C9, or CYP2C19-mediated metabolism are taken together with EEDS. Characterization of metabolic profiles of specific herbal drugs could help consumers and medical specialists to use them safely as a complementary and alternative medicine.

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PMF-1 Methylation Is a Prognostic and Predictive Biomarker of Sunitinib Response in Patients with Kidney Cancer

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Hypothesis: Sunitinib represents a reference treatment of metastatic kidney tumors. However, it is not clear yet which patients are more susceptible to respond. We hypothesize polyamine content may impact on sunitinib response.

Objectives: The aim of this study was to evaluate the role of polyamine modulated factor-1 (PMF-1) methylation at predicting clinical outcome in renal cancer and therapy response in metastatic cases treated with sunitinib.

Methods: In a retrospective design, PMF-1 methylation was analyzed on 67 tumors of patients with localized disease and no evidence of disease during follow-up (n=25) (Group 1), and 42 patients treated with sunitinib, from which Group 2 had non-metastatic disease at diagnosis (n=23), and Group 3 showed metastatic disease at diagnosis (n=19). PMF-1 methylation was assessed by methylation-specific polymerase chain reactions (MS-PCR). Progression free survival, disease-specific survival and overall survival rates were analyzed using univariate and multivariate Cox regression analyses.

Result: Among the 67 cases analyzed, 7 of them recurred (10.4%), 34 progressed (50.7%), and 25 died of disease (37.3%). PMF-1 methylation was found in 42/67 (62.7%) tumors and correlated to Fuhrman classification (p=0.030). Taking all patients, PMF1 methylation was a prognostic biomarker for progression (p=0.003), disease-specific survival (p=0.025) and overall survival (p=0.016) in univariate and multivariate analyses. PMF-1 methylation was the only factor among clinical variables predicting sunitinib response for progression-free survival (p=0.026). Moreover, it remained as an independent predictive biomarker for disease-specific (p=0.009) and overall survival (p=0.021).

Conclusions: Thus, epigenetic analyses revealed that the methylation status of PMF-1 associated with the clinical outcome of patients with advanced kidney tumors as a prognostic and predictive marker for patients undergoing sunitinib treatment. An unmethylated PMF-1 identified patients with shorter progression free survival, disease-specific survival, and overall survival. Thus, assessing the methylation status of PMF-1 may serve to distinguish patients responding to sunitinib.

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Synergistic and Additive Therapeutic Effects of Pectins to Cisplatin in Bladder Cancer Cells

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Hypothesis: Cisplatin-based combinations represent the standard chemotherapy for patients with metastatic bladder cancer. It remains unknown which patients respond or may become resistant to cisplatin. We hypothesized that pectins would enhance the anticancer effect of cisplatin in vitro in bladder cancer cells.

Objectives: The objective of this study was to evaluate the combined effect of a known galectin-3 inhibitor, PectaSol-C modified citrus pectin (MCP), and 6 novel pectin compounds purified from olives and cisplatin in bladder cancer cells

Methods: The anticancer effect of MCP and novel purified pectins was tested in vitro in 5 bladder cancer cells alone and combined to cisplatin. Viability proliferation, migration, and IC50 were evaluated. Secretion of galectin-1 and galectin-3 upon pectins and cisplatin exposure was determined using enzymeimmune analyses. Changes in bladder cancer signaling pathways upon pectins and cisplatin exposure were determined by Western blot analysis.

Result: MCP and olive purified pectins diminished cell proliferation, viability and migration of bladder cancer cells, enhancing cisplatin cytotoxic effects. IC50 analyses identified squamous ScaBER cells as sensitive cells to pectins as compared to resistant bladder cancer cells. Galectin-1 and galectin-3 secretion increased upon exposure of bladder cancer cells to pectins alone or combined to cisplatin. Western blot analysis releaved differential changes in galectins and p53 expression among sensitive and resistant cells upon pectins and cisplatin exposure.

Conclusions: The combination of MCP with cisplatin synergistically inhibited cell proliferation and migration in bladder cancer cells. Pectins exposure modified the expression of in bladder cancer signaling pathways, likely via galectins interaction. Further studies confirming these observations suggesting the therapeutic utility of pectins in animal models of bladder cancer are warranted.

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A Reflex Algorithm for Identifying Presence of Extra-Colonic Cancers in False-Positive CRC Screened Subjects

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Hypothesis: A reflex algorithm approach based on various cancer-associated soluble protein biomarkers may aid in identifying extra-colonic cancers in subjects screened false positive by a primary CRC screen with a subsequent negative colonoscopy.

Objectives: To develop a reflex algorithm utilizing blood-based protein biomarkers and clinical parameters to identify subjects who may have an elevated risk for extra-colonic cancer after a positive primary screen and no findings at colonoscopy.

Methods: Using the CRC screening trial Endoscopy II, AIM (Adaptive Index Modeling) was used to develop a primary algorithm (to identify persons at higher risk for CRC or high risk adenoma lesions) and a reflex testing algorithm (to identify subjects at risk for the presence of extra-colonic cancer in subjects with a false positive result from the primary algorithm). Using this dual approach, patients identified as primary algorithm positive would be recommended for a screening colonoscopy and negative screening subjects would be followed up at the next interval. Screen- positive patients negative upon colonoscopy would be reflexed to a secondary panel to determine likelihood for presence of an extra-colonic cancer. The clinical performance of the secondary algorithm was evaluated by estimating the net sensitivity, specificity, PPV, NPV, as well as the rate of unnecessary colonoscopies.

Result: The developed reflex algorithm identified extra-colonic cancer at 81% sensitivity and 77% specificity for males, and at 59% sensitivity and 93% specificity for females. Utilizing the dual algorithm approach, approximately 9% of subjects predicted to be negative by the primary algorithm would have missed a necessary colonoscopy, whereas 90% of screen-negative subjects would have avoided unnecessary colonoscopies. Among primary screen-positive subjects, 1/3 would have been correctly recommended to colonoscopy as CRC was found upon colonoscopy. Although predicted to be positive by the primary algorithm, 2/3 would have no findings. Reflexed to the secondary algorithm resulted in approximately 25% of the subjects being misclassified as having extra-colonic cancer.

Conclusions: The results support the potential usefulness of employing multiple algorithms to address key clinical decision making needs.

Funding Source: Abbott Labs

Targeting Cellular Cholesterol Might Be a Therapeutic Strategy for Breast Cancer Bone Metastasis

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Hypothesis: Cancers are the most complex diseases, and also the second most leading cause of morbidity and mortality of patients worldwide. Epidemiological studies documented a positive association between many environmental factors/biological parameters and cancer risk. It was hypothesized that high cellular cholesterol positively influences breast cancer bone metastasis.

Objectives: 1. To find the link between serum cholesterol and cancer mortality, 2. To examine whether cholesterol depleting/lowering drug blocks breast cancer induced osteolytic metastasis.

Methods: Statistical analysis of worldwide data of 166 countries, TRAP assay/staining for measuring osteoclast activity, RT-PCR analysis for determining the expressions of various osteoclastogenic genes.

Result: Our statistical analysis of worldwide data of 166 countries, for the first time, found an existence of a positive correlation between serum average total cholesterol and overall cancer mortality rate (CMR), and also with different anatomical site specific CMRs in lung, bladder, ovarian, breast and pancreatic cancers of a country. In this study, it was observed that treatment of pre-osteoclast RAW264.7 cells with conditioned medium (CM) of breast cancer MCF-7 cells showed an enhancement of osteoclast activity, evidenced by TRAP assay/staining, with simultaneous increment of expressions of various osteoclastogenic genes (e.g., TRAP, Cathepsin K and NFATc1). Simvastatin treatment blocked the breast cancer-induced TRAP activity and the expressions of these osteoclastogenic genes in RAW264.7 cells. In addition, simvastatin showed an inhibition of osteoclastogenic ligands CSF-1 and RANKL expression in MCF-7 cells.

Conclusions: Thus, these findings unravel a molecular mechanism involved in simvastatin-mediated inhibition of breast cancer-driven osteoclast activity, responsible for osteolytic metastasis.

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Cold Environment is a Risk Factor for Cancer

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Hypothesis: Recent findings suggest that both mutation and epigenetic changes cooperate to initiate cancer progression and development. Moreover, the epigenetic methylation at CpG islands may not only silence the gene expression but also promotes the genetic mutation and genetic instability. Thus, the extrinsic factors which modulate methylation or epigenetic changes might have a predominant role in cancer incidence and development.

Objectives: The objective of this study is whether cold exposure may alter cancer incidence and/or deaths.

Methods: Univariate analysis was done between average annual temperature (AAT) and cancer incidence rate (CIR) or cancer mortality rate (CMR). MTT, cell count, cell migration and colony formation assay were performed to see the effect of cold exposure on various cancer cell lines. Intracellular cholesterol was measured by colorimetric assay kit. RT-PCR was performed to check the effect of cold on various gene expressions related to EMT markers and genes which regulate cellular cholesterol level.

Result: Univariate analysis of 188 country data found an existence of a negative correlation between AAT and CMR for all anatomical sites (AAS) and many site specific cancers such as breast, ovary, bladder etc. Similarly, a negative correlation was observed between AAT and CIR for overall (AAS) and site specific cancers including breast, ovary, thyroid, bladder etc. for female population when we had analysed 2910 counties data of USA. This inverse association exists in all ethnicities/casts of USA female population. Further findings suggested that cholesterol could be a mediator of cold-induced cancer mortality. Our preliminary cell culture based experimental observations showed that cold shock increased proliferation and migration of various cancer cells such as breast cancer MCF-7 and MDA-MB-231 cells with simultaneous increment of intracellular cholesterol. Cold shock also enhanced the colony formation of breast cancer cells. RT-PCR analysis found that cold shock may change molecularmarkers

Conclusions: These epidemiological findings unravel a novel link between cold environment, cholesterol and cancer. Cell culture based studies support that cold exposure might augment cancer growth and migration presumably by increasing cellular cholesterol of cancer cells.

Funding Source: N/A

Omega-3 Fatty Acid Disrupts Osteoblastic Potential of Breast Cancer Cells

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Hypothesis: Mammary microcalcifications (MC), important radiological indicator of breast cancer have been categorized in two types; calcium oxalate (type I) and hydroxyapatite (type II). Recent studies suggested a positive association with MC (type II) and cancer malignancy. However, the mechanism that drives microcalcification process is poorly understood but can be correlated to the property of osteoblastogenesis. Thus, the disruption of osteoblastic potential might be a therapeutic strategy for prevention of microcalcification in breast cancer tissue.

Objectives: The principle aim of present study was intended to investigate whether breast cancer cells possess osteoblastic potential by examining expression of various osteoblastic markers as well as bone matrix proteins and concomitantly to investigate effect of “omega 3 fatty acids (ω-FAs)” on osteoblastic potential of breast cancer cells.

Methods: Two breast cancer MCF-7 and MDAMB231 cell lines were used in this study. RT-PCR analysis was done to check the expressions of different osteoblastic markers and transcriptions factors. ALP and Alizarin staining was done to examine ALP activity and mineralization respectively in both cultured cells as well as in formalin fixed paraffin embedded breast cancer tissue slides.

Result: RT-PCR results showed the expression of osteoblastic markers (ALP and ON) and osteoblastic transcription factors (Runx2 and Msx2) in both MCF-7 and MDAMB231 breast cancer cells. Moreover, it was found that breast cancer cells showed ALP activity and nodule formation (an indicator of calcification) as evidenced by ALP and Alizarin staining respectively. Our studies also documented that breast cancer tissues contained more ALP activity and mineralization as compared to benign tissues. We also studied effect of DHA on osteoblastic potential of both breast cancer cells, and found that DHA treatment was able to inhibit expression of osteoblastic markers (ALP and ON) and osteoblastic transcription factors (Runx2 and Msx2) in both MCF-7 and MDAMB231 breast cancer cells. Furthermore, we confirmed these results by ALP and Alizarin staining in breast cancer cells and found that DHA has inhibitory potential to suppress osteoblastic properties of breast cancer cells.

Conclusions: In conclusion, present study illuminates the osteoblastic potential of breast cancer cells and tissues. Also, this study first time reported that ω-FA can attenuate osteoblastic phenotype of breast cancer cells and can become promising treatment to target breast microcalcification.

Funding Source: N/A

Alteration of Iron and Cholesterol for Neuroblastoma Treatment

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Hypothesis: Identification of a unique relationship between iron and cholesterol in treatment-resistant neuroblastomas could provide the basis for development of new treatment options.

Objectives: To examine the relationship between iron and cholesterol metabolism in neuroblastomas to determine whether alteration of either would provide a treatment option for those that are drug resistant.

Methods: The HFE genotype of neuroblastoma cell lines was determined by direct sequencing of the PCR product. Cellular iron concentrations were determined using the QuantiChrom Iron Assay Kit and cholesterol content using the Biovision cholesterol quantification kit. Expression of proteins related to iron or cholesterol metabolism in neuroblastoma cells was determined by standard western blotting procedures. HFE or C282Y HFE siRNA (Ambion) was used to determine whether expression of C282Y HFE affects the concentration of cellular iron and cholesterol as well as their metabolism. To determine the association between iron and cholesterol, we treated cells with the iron chelator DFO or Simvastatin, a HMGCoA reductase inhibitor, or iron (ferrous sulphate). The anti-tumor effect of the agents identified as most effective in the in vitro studies was determined using a mouse neuroblastoma tumor model.

Result: We found that drug resistant neuroblastoma cells have alterations in expression of genes encoding proteins involved in iron and cholesterol metabolism relative to drug susceptible cells. To address the question of whether alterations in either iron or cholesterol levels affected viability of drug resistant neuroblastoma cells they were exposed to iron chelators and cholesterol decreasing agents. Results of in vitro experiments indicated that simvastatin, an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase, combined with U18666A, an inhibitor of the last step in cholesterol synthesis, were cytotoxic to drug resistant cells as was the iron chelator di-2-pyridylketone-4-cyclohexyl-4-methyl-3-thiosemicarbazone (DpC). In vivo, the anti-tumor effect of simvastatin administered orally was minimal.

Conclusions: Our results indicate that it may be possible to develop methods to use certain iron chelators and/or cholesterol decreasing agents in the treatment of small size of drug resistant neuroblastomas.

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Mir-26 Regulates Cell Migration and Motility by Targeting Pathway Associated Genes

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Hypothesis: Cell migration is a critical biological process that is required during embryonic development, wound healing, immune responses. Aberrations in regulating cell migration are associated with various diseased physiology including metastasis, autoimmune disorders, etc. Evidently, this process is tightly regulated at multiple levels. MicroRNAs (miR) are small, non-coding RNA that post-transcriptionally silence mRNAs by binding to their 3′UTRs. We therefore hypothesize that miRs regulate cell migration and motility.

Objectives: Screen various miRs for their potential role in modulating cell migration and study underlying mechanism.

Methods: Various cancerous and non-cancerous cell lines were cultured. Expression of eight different miRs was checked by qRT-PCR. High expressing miRs or control mimics were transiently transfected using Lipofectamine 2000. After 24 h, cell migration was assessed by performing in vitro wound closure assays over the period of 48 h. Bioinformatics analysis of predicted pathway and gene target was performed using various algorithms. We cloned 3′UTR of PLCB1 in a reporter construct and performed dual luciferase assays to validate miR-26 and PLCB1 interaction.

Result: Among the tested miRs we noticed high expression of miR-24, miR-26a, miR-30b, and miR-101a in all the cell lines. These miRs were tested for their impact on cell migration. We noticed that miR-26 had most significant impact on cell migration in the wound closure assays. In silico analysis revealed various pathways targeted by miR-26. These included cytoskeletal rearrangement, cell migration, PI3K-Akt pathways, etc. Using dual luciferase assays we validated miR-26 mediated downregulation of phospholipase C, beta 1 (PLCB1).

Conclusions: We screened multiple miRs and identified miR-26a as a potential modulator of cell migration in cells lines of multiple origins.

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miRNA-Target Interaction Reveals Cell-Specific Post-Transcriptional Regulation in Mammalian Cell Lines

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Hypothesis: In our previous study we noticed dramatic transcriptome wide changes in Dendritic cells (DC) challenged with periopathogen derived LPS while, macrophages (Mϕ) under similar challenge exhibit comparatively less significant changes. mRNA expression is governed post-transcriptionally by microRNA (miRNAs). Upon binding to their target mRNAs, miRNA can either degrade transcript or suppress translation. We also observed that miRNA or control mimics transfected DC show remarkably altered mRNA expression of various genes examined while, only few were impacted in Mϕ. We hypothesize that cells may differ in their mode of miRNA regulation.

Objectives: To examine the cell specific mode of miRNA target regulation in various mammalian cell lines using identical miRNA binding site.

Methods: Mammalian cell lines were procured from the ATCC. Transfection efficiency was assessed by using pGLO MAX GFP expression plasmid. Cells were transfected with pRL-TK-let7a wild type (WT) or mutant (MT) and reporter gene expression was determined by dual luciferase reporter assays. Firefly expressing plasmid served as transfection control. Total RNA was also isolated and expression of renilla and firefly luciferase mRNA was detected by qRT-PCR. GAPDH was used as internal control. Incorporation of miRISC (miRNA-RNA induced silencing complex) associated miRNA target was quantified by immunoprecipitating Ago-2 and then quantifying the levels of WT and MT renilla mRNA by qRT-PCR.

Result: Dual luciferase assays show that compared to mutant (mut), the reporter gene containing wild type (wt) let-7a binding sites was efficiently suppressed upon transfection in various cell lines. Importantly, the strength of miRNA regulation varied across the cell lines. Total RNA analysis demonstrates that wt renilla mRNA was expressed to similar or higher levels compared to mut suggesting that translation repression is a predominant mode of miRNA regulation. Nonetheless, transcript degradation was observed in some cell lines. Ago-2 immunoprecipitation show that miRNA repressed renilla mRNA are associated with functional mi-RISC.

Conclusions: Given the immense potential of miRNA as a therapeutic option, these findings highlight the necessity to thoroughly examine the mode of mRNA regulation in order to achieve the beneficial effects in targeting diseased cells viz., cancer cells, aberrant immune cells.

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Validation of an Algorithm Using Tumor Markers, Clinical Data and Image Techniques to Help in The Diagnosis and Histology

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Hypothesis: Lung cancer (LC) is the most frequent and fatal human cancer. Its prognosis is directly related to early diagnosis. We have previously identified six serum tumor markers (TM) (CEA, CA15.3, SCC, CYFRA 21-1, NSE and ProGRP) related to the presence of lung cancer (LC).

Objectives: To (1) validate the efficiency of 6 tumor markers for LC presence (2) validate an algorithm that help in stablish risk of lung cancer and the histological subtype (3) to evaluate the improvement of this algorithm using othr 3 new tumor markers (CA 19.9, CA 125 and Tag-72).

Methods: We investigated the diagnostic utility of a combined panel of six serum TM (CEA, CA125, SCC, CYFRA 21-1, NSE and ProGRP) in 4,296 consecutive individuals referred to our institution because of the clinical suspicion of LC. Using standard clinical procedures, LC was confirmed in 2.681 (62,4%) patients (2,216 with NSCLC and 465 with SCLC) and excluded in 1,615 individuals (37.6%).

Result: (1) validated the previously reported performance of each individual TM; (2) showed that their combined assessment had a better sensitivity, specificity, NPV and PPV (88.5%, 82%, 83.7% and 87.3%, respectively) that each TM considered individually, and that it increased the diagnostic performance (AUC) of a clinical model that included tumor size, age and cumulative smoking exposure; (3) in patients with radiographic nodules <3cm, the NPV of the TM panel was 60.8%, hence providing some support for a more conservative diagnostic approach; and, (4) identified two TM (NSE and ProGRP) that differentiate the risk of NSCLC from that of SCLC. We validate the algorithm with the sensitivity previously indicated and with a specificity of 98% using serial tumor marker determination. Likewise, the introduction of CA 125, CA 19.9 and TAG-72 do not improve the sensitivity and specificity obtained with the 6 tumor markers described.

Conclusions: The combined panel of serum TM investigated here is a more accurate marker for LC presence that these same TM considered individually. Sensitivity and specificity suggest their possible utility as help in the diagnosis. Likewise, the six serum TM show a significant capacity to differentiate NSCLC and SCLS, which may require different therapeutic strategies.

Funding Source: N/A

Tumor Markers Use for The Differential Diagnostic of Effusions

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Hypothesis: Effusions are a frequent reason for clinical consultations and about 5 – 20% of all cases are of neoplastic origin. Between 24 and 50% of patients with disseminated cancer develop pleural effusions, with about 100,000 new cases per year in the USA. These data clearly show the importance of having an efficient method for diagnosis (primary-metastatic) and differential diagnosis of patients with effusions. Cytology of the fluid is the main technique used, but unfortunately over 30% of malignant effusions display negative cytology.

Objectives: This study presents a guideline for the diagnostic possibilities of malignant effusions, using tumor markers. This guideline describes the main problems in determination and interpretation of tumour markers in fluid effusions and possible solutions for them.

Methods: There have been important discrepancies reported in relation to the cut-off, for example from 3 ng/ml to 100 ng/ml with CEA. To avoid this problem, the simultaneous determinations of tumor markers in effusions and serum have been suggested by our group in five publications including more than 1000 patients with pleural effusion or ascites. If concentrations are higher in the effusion compared to serum it indicates local production and indirectly metastases.

Result: Tumor marker sensitivity is high 70-80 % depending on the chosen combination with a high specificity (>98%) in both patients with negative or positive cytology in the fluid.

Conclusions: Tumor marker determination in fluid and in serum may help in the differential diagnosis of effusions, in both patients with positive or negative citology.

Funding Source: N/A

Are Tumor Markers Useful in Clinical Practice According to Personalize Medicine? From The Lab to the Bed.

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Hypothesis: General criteria indicate that tumor markers are habitually not used in diagnosis because sensitivity and specificity is low. Evidently tumor marker sensitivity, changing according tumor origin, is related to tumor stage with higher serum concentrations in advanced stages. However, tumor marker may be useful to discriminate patients with high risk of malignancy, where other technique may be used to confirm or not the suspicious as happen in prostatic cancer, colo-rectal cancer or ovarian cancer.

Objectives: Evaluate the criterias that are related to the possible use of tumor markers for diagnosis or the histological diagnosis

Methods: Our group use 3 criterias to evaluate tumor markers, first to exclude the main source of false positive results (use a second cut-point in these patients), second to evaluate the serum levels of these tumor markers, selecting some cut-points that indicate with a high probability (>95%) cancer and finally in those cases with doubts, use serial determination.

Result: Our results in 6423 patients with paraneoplastic syndromes or symptoms suggestive of advanced cancer show a high sensitivity (76%) and specificity (86% in single determination, 99% in serial determination). Similar results were found to select high risk of cancer in patients with suspicious signs of colorectal-cancer (anemia, fecal blood positive), lung nodules, abdominal masses, etc.

Conclusions: In summary the correct use of tumor markers is useful in the clinical practice helping doctor in making diagnosis.

Funding Source: N/A

How Biomarker Results from the Laboratory Can Support the Clinician

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Hypothesis: The algorithm, ROMA (Risk of Ovarian Malignancy Algorithm) is suggested to improve the sensitivity and specificity of CA 125 & HE4 by combining both tumor markers in patients with abdominal masses.

Objectives: To understand clinical applications of tumor markers in ovarian cancer by looking at the differential diagnosis of abdominal masses, as well as in the follow-up of patients with ovarian or endometrial cancer.

Methods: We have studied prospectively all these tumor markers as well as CA 19,9 in 564 healthy women, 885 patients with benign diseases, 183 patients with ovarian cancer, treated and without evidence of disease and 226 patients with ovarian cancer.

Result: The results clearly show that HE4 had a significantly higher specificity than CA 125, mainly in premenopausal women with false positive results in less than 5% of the patients in contrast to the 28% found with CA 125. Likewise, HE4 had a better sensitivity than CA 125 in ovarian cancer, mainly in the early stages. Histology is clearly related to tumor marker sensitivity being HE4 and CA 125 the best tumor markers in non-mucinous tumors and CA 19.9 in mucinous tumors. Tumor markers are useful in the differential diagnosis, independent of ultrasonography. HE4 and CA 19.9 were the best combination in premenopausal women and all tumor markers, including ROMA in postmenopausal women.

CA 125 and HE4 serial determination is a useful tool in the early diagnosis of recurrence and in therapy monitoring. CA 125 was correlated with tumor response in 83%, HE-4 in 85% and any of them in 92% of our patients. Similar results were found when mucinous tumors were excluded: any of the tumor markers w

Conclusions: Tumor markers are useful in helping the differential diagnosis of abdominal masses as well as in therapy monitoring of ovarian cancer

Funding Source: N/A

Evaluation of Biochemical Parameters in Selenium-Supplemented Infant Patients Using Non-Linear Optical Method (Z-SCAN)

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Hypothesis: Cancer is a disease that affects the metabolism causing an imbalance in the patient's health. On the other hand, malnutrition, presented by cancer patients can be caused by disease and systemic treatment. Biochemical parameters evaluated in cancer patients cannot be detected as altered due to conventional laboratory methodology used (spectrophotometry). Among the methods that can be used for biochemical analysis in the blood, there is the nonlinear optical method known as Z-Scan.

Objectives: To evaluate biochemical markers (total cholesterol, triglycerides and glucose) in children with leukemia (ALL / AML) and solid tumors (STU) after selenium supplementation using nonlinear optical method Z-Scan.

Methods: Children and Adolescents with ALL / AML and STU of both sexes were submitted to a clinical trial randomized phase II, double-blind with intersecting groups (selenium versus placebo). Blood samples for determination of biochemical parameters were obtained before supplementation of placebo/selenium, after 60 and 120 days of treatment as group patients belonged (placebo or selenium). Colorimetric enzymatic reaction for glucose, total cholesterol and triglycerides was performed on sample port adapted for determining the Z-Scan.

Result: 26 patients were included in the study. The average age of all patients was 9.48 years. Regarding glucose parameters, total cholesterol and triglycerides determinate by Z-Scan method there was a statistical tendency to changes in glucose concentrations when we compare the times 60-120 days ($p = 0.006$) and 1-120 days ($p = 0.073$). Interestingly, such interference supplementation on blood glucose concentration was not displayed by the conventional method (spectrophotometry) in the patients studied.

Conclusions: It is possible to use nonlinear optical method Z-Scan as an analysis tool of biochemical parameters in cancer pediatric patients supplemented with selenium.

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Correlation between Homocysteine Levels, Derivative Products of Lipid Peroxidation and Clinical Laboratory Variables in Prostate Adenocarcinoma Patients during Chemotherapy

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Hypothesis: In prostate cancer should be a balance in the production of free radicals and a decrease in antioxidant capacity can disturb this balance and act to promote prostate carcinogenesis. Thiobarbituric acid reactive substances (TBARS) is a marker of lipid peroxidation of macromolecules. Thus can result in a disorder in homocysteine metabolism and their co-factors (Vitamin B12 and folic acid).

Objectives: To evaluate the levels of homocysteine and its cofactors, thiobarbituric acid (TBARS) and correlate the results of clinical laboratories indicators in patients with prostate cancer undergoing chemotherapy

Methods: 38 patients with prostate adenocarcinoma were included. Samples were collected at diagnosis and 6 months of the treatment. Homocysteine, Vit B12, folic acid, total PSA, E-cadherin, MMP-13 and TBARs were evaluated following the good practice in clinical laboratory analysis.

Result: It was observed that TBARS at diagnosis (Time 1) and after 06 months (Time 2) levels showed equivalent median and confidence interval (CI) 95% 0.02 (0.02 to 0.03), respectively. The median and the 95% of confidence interval (CI) of the others parameters were 11.4 (8.93 to 15.09) for homocysteine levels, 320.0 (216.09 to 533.38) for vitamin B12, 3.6 (2 , 20-4.20) for the folic acid, 15.71 (9.19 to 42.89) to E-cadherin, 0.04 (0.03 to 0.07) for MMP13, 0.12 (0, 04 to 2.4) for PSA at diagnosis (Time 1) and 0.07 (0,04- 0,2) for PSA (Time 2).Furthermore, there was a positive moderate and significance correlation between TBARs (Time 1) and Homocysteine (Spearman 's rho = 0.501, p = 0.033).

Conclusions: There was correlation between TBARs and homocysteine concentrations in samples collected at diagnosis. It is suggested that dosage of TBARs can be a biomarker of unba lance in the carcinogenesis at diagnostic in prostate cancer patients.

Funding Source: N/A

Frequency of Gene Polymorphisms C677T, A1298C and G1793A in Gene Methylenetetrahydrofolate Reductase (MTHFR) in Patients

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Hypothesis: Bladder cancer is the second malignancy with the highest incidence in the genitourinary tract and the sixth most common type of cancer worldwide. The incidence of bladder cancer increases with age, the maximum effect is reached at the age of 60 years. Cancer is the result of a genetic alteration that is capable of making proto-oncogenes and / or oncogenes are suppressed and / or overexpressed. Polymorphisms in genes of enzymes that participate in DNA methylation process, for example, MTHFR, can modulate gene regulation, since it is responsible for metabolize folate and methyl groups available for the organism.

Objectives: To determine the presence or absence of polymorphisms in the gene MTHFR enzyme in patients with bladder cancer relating to the diagnosis.

Methods: The urinary and plasma DNA were extracted using the GFX TM kit (Amersham Pharmacia Biotech). The urinary DNA was quantified with Spectrophotometer GeneQuant RNA / DNA Calculator Amersham Pharmacia Biotech. Detection of the C677T polymorphism (rs1801133), A1298C polymorphism (rs1801131) and G1793A (rs2274976) in the MTHFR gene was performed using the TaqMan system for real-time PCR using the Real Time PCR SetpOne platform (Applied Biosystems®).

Result: It was only detected the presence of A1298C polymorphism in the gene. Homozygous allele 1 was observed in 7% of sample. While in homozygous allele 2 was present in 36% of samples. The heterozygosity was demonstrated in 43% of sample and 14% of samples gave indeterminate results.

Conclusions: It is suggested that the samples with A1298C polymorphism in the region have predisposition to the development of urothelial carcinoma serving as a classificatory diagnostic mode. Is possible to determine polymorphism in sedimentary urine samples.

Funding Source: N/A

COX 2 Expression and Relationship with Anatomopathological Data in Patients with Prostate Cancer

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Hypothesis: In many types of cancer is found an overexpression of cyclooxygenase 2 (COX 2). The COX2 is linked to cancer aggressiveness parameters, tumor size, positive nodal status and lower survival. It is also related to angiogenesis and apoptosis resistance. Therefore, our hypothesis is that the COX2 is expressed in greater quantities in patients with prostate cancer and that this marker could aid in the diagnosis and prognosis of the patient.

Objectives: To observe the expression profile of the COX2 gene in patients with prostate cancer clinic FMABC.

Methods: 15.0 mL of peripheral blood were collected from 27 patients and 25 healthy men. RNA extraction and cDNA synthesis were performed according to the recommendations of the respective kits: QIAamp RNA blood mini and Superscript II reverse transcriptase RNAse. The quantitative Real Time-PCR was performed with the aid of specific primers COX2 and the endogenous gene GAPDH.

Result: The mean age of patients was 66 years (77.77%: localized disease, 7.41%: locally advanced disease, 7.41% and 7.41 metastatic disease had not informed his staging). The average of COX2 expression in the five collections of patients with prostate cancer was 2.01 respectively; 4.36; 5.09; 2.49 and 1.72 while the control group had an average of 0.502.

Conclusions: The group of patients with prostate cancer had an average higher expression of COX2 diagnosis than the control group during the treatment expression increased and decreased at the end of treatment. This can have an important significance in the diagnosis and prognosis of the disease requiring retesting with an "n" most samples.

Funding Source: N/A

The Cadherin-1, Type-1 (CDH1) Gene is Down Regulated by High Nitric Oxide Levels in Head & Neck Squamous Cell Carcinomas (SCC)

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Hypothesis: High Nitric Oxide (HNO) Levels in Head and Neck Squamous cell carcinomas cause down regulation of genes that lead to metastasis potential.

Objectives: Nitric Oxide is a free – radical molecule that all cells in our body produce. Nitric Oxide levels at a normal level help cells to be able to communicate and transmit signals throughout the body. High levels of Nitric Oxide in Cancer patients are considered to be cytotoxic while low levels of Nitric Oxide in Cancer patients are considered cytostatic. Overexposure of HNO levels in cells cause protein coding genes to be down regulated that play a key role in cell adhesion, cell signaling and cell communication. In order to study more on the relationship between HNO Levels and metastatic potential, High Nitric levels were presented in four of the H&N SCC cell lines. Adapting of HNO levels in H&N SCC cell lines caused down regulation of CDH1 in all four of H&N SCC cell lines that were observed. One of the causes of carcinogenesis is due to abnormalities in tumor suppressor protein coding genes. In this case, CDH1 was found to be down regulated. CDH1 gene provide manuals for producing a protein that codes for Epithelial Cadherin or E-Cadherin. E-Cadherin regulate cell- adhesion, cell proliferation which plays an important role as tumor suppressor genes.

Methods: The H&N SCC cell lines SCC016, SCC040, SCC056, and SCC114 were studied by adjusting them to HNO cancer cell lines. Nitric Oxide levels were increased to HNO by DETA-NONOate. Also, DNA microarrays was performed in order to compare the control cell line and and HNO cell line.

Results: Adaption of HNO levels in H&N SCC cell lines caused CDH1 genes to be down regulated.

Conclusions: Adapting HNO levels to the H&N SCC cell lines that were studied causes CDH1 to be down regulated**.** Down regulation of CDH1 means that the gene expression is reduced or decreased. Therefore, causing it to be increase in metastatic potential. More research can be done on studying about the relationship between carcinogenesis and CDH1. Other studies can be done by looking at the relationship between different Nitric Oxide levels and CDH1.

Funding Source: VA Merit Review

Cell Division Cycle Protein 27 (CDC27) Is Down-Regulated In High Nitric Oxide-Adapted Breast Cancer Cell Lines

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Hypothesis: Adaptation of breast cancer cell lines to high nitric oxide (NO) will result in decreased expression levels of genes involved in the regulation of cell cycle arrest.

Objectives: A key feature of cancer is the progression through the cell cycle in an uncontrolled manner. Adaptation to high NO levels results in an observed down-regulation of important genes that are involved in cell cycle regulation. Breast cancer cell lines were adapted to high NO (HNO cancer cells), and then compared to their corresponding parent cell lines. Expression of the CDC27 gene was observed to be down-regulated in HNO cancer cells from the breast cancer cell lines. CDC27 codes for a subunit of the anaphase-promoting complex/cyclosome (APC/C), which is an important regulator of the spindle checkpoint in cell division.

Methods: Three breast cell lines were studied: Hs578t, MCF7, and T47D. Using an NO donor, DETA-NONOate, the cell lines were adapted to high levels of NO. A genome-wide gene chip experiment was used to assess the expression levels of genes between the HNO and corresponding parent cell lines.

Results: The CDC27 gene was down-regulated in the HNO cancer cells of the Hs578t, MCF7, and T47D breast cancer cell lines.

Conclusions: Exposure to high levels of NO results in down-regulation of the CDC27 gene in HNO cells of breast cancer compared to their parent cell lines. Further studies with varying expression levels of CDC27 may be helpful in understanding its impact on cell proliferation when comparing HNO cancer cells to their parent cells. It might also be helpful to search for expression levels of other genes that play an important role in cell cycle progression, and, thus, further explore the effects of nitric oxide on cell proliferation.

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Tumor Stem Cell (TSC) Creation via High Nitric Oxide (HNO) Adaptation in Adenocarcinomas (ACs)

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Hypothesis: Adaptation to high concentrations of nitric oxide (HNO) causes ACs to exhibit an aggressive phenotype, similar to that of cancer stem cells. This occurs because the expression of certain pathways, including cellular respiration, is changed.

Objectives: Adaptation to HNO results in ACs demonstrating a more aggressive phenotype in comparison to non-adapted cells. These HNO-adapted cancer cells behave akin to cancer stem cells. The molecular and cellular processes by which this HNO-induced transformation occurs remains unknown. Understanding these mechanisms will allow for potentially new drugs to be developed to specifically kill TSC.

Methods: Five human AC cancer cell lines (Lung: A549; Breast: BT20, Hs578, MCF7, and T47D) were studied. The cell lines were adapted to HNO by gradual exposure to increasing quantities of DETA-NONOate, an NO donor. mRNA isolated from both the parent and HNO cell lines were tagged with red/green fluorescent markers and used to compete in a full human genome gene chip analysis experiment.

Results: HK1 and COX7C were consistently up-regulated and LDHB, LDHBP, PDHA1, AC004490.1, and LSM7 were consistently down-regulated.

Conclusions: High concentrations of nitric oxide significantly affected the cellular respiration pathways in ADC cell lines. PDHA1, pyruvate dehydrogenase alpha 1, is primarily responsible for catalyzing the transform of pyruvate to acetyl-CoA, connecting glycolysis to the Krebs Cycle. Its down-regulation suggests that AC cells use a nonstandard pathway for adenosine triphosphate (ATP) creation.

Funding Source: VA Merit Review

Pseudogenes May Play a Role in Cancer Stem Cell Formation via Increased Nitric Oxide (NO) Exposure

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Hypothesis: With HNO, CeRNA and microRNA are affected such that pseudogenes (and coding analogs) are over expressed in cancer cells.

Objective: Although pseudogenes were traditionally thought to have no functional significance, evidence suggests that pseudogenes may contribute to the development of cancer as CeRNA (competing endogenous) mediates microRNA sequestration. A parallel dysregulation of the BRAF gene and its pseudogene have been reported. Specifically, high levels of the BRAF pseudogene (entailing a high amount of the regular BRAF gene) lead to the development of aggressive malignancies.

Methods: Gene chip analysis of ten HNO adapted cell lines (Squamous cells: SCC-016, SCC-040, SCC-056, SCC-114, SCC-116; Adenocarcinomas: A549, BT20, Hs578, MCF7, and T47D) was carried out. Known pseudogenes were identified in each line, as well as their coding counterparts.

Results: The adenocarcinoma cell lines had no up regulated pseudogenes, while they had the following down regulated pseudogenes: RP6-159A1.2, RP11-255N24.3, AC004490.1, LDHBP, RP11-572H4.2. The squamous cell carcinomas (SCCs) had the following up regulated pseudogenes: RPL37AP1, AC138972.1, RP11-641D5.1, AC005534.6, AC022431.1, RPL26P12, and they had these down regulated pseudogenes: RP6-159A1.2, RP11-255N24.3, RBMXP1, RP11-20O23.1, RP11-551G24.2. All cell lines adhered to the hypothesis that an increase in a pseudogene expression also had an increase in the corresponding gene (with the exception of the adenocarcinoma cell lines).

Conclusions: The high level of pseudogenes could be due to low levels of microRNA; low expression of microRNA could then be due to high levels of CeRNA. In cases when the pseudogenes increase in expression (possibly due to HNO interference) they, like BRAF, take the functionality of CeRNA which in turn decreases microRNA expression. Although a pseudogene may not have any direct translational significance, it can act as CeRNA to facilitate the over expression of the coding gene in a feedback loop.

Funding Source: VA Merit Review

High Nitric Oxide Adapted Tumor Cells Ensure Survival by Up or Down Regulating Specific Genes within the Apoptosis Pathway

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Hypothesis: Squamous, Breast, and Lung tumor cells adapted to high levels of nitric oxide (HNO) will exhibit up or down-regulation in key points of the apoptosis pathway to prevent apoptosis from occurring.

Objectives: Apoptosis is a method of programmed cell death that occurs to remove cells that can damage the organism. The apoptosis inducing factor (AIF) gene has been found to play an important role in the regulation of the apoptosis pathway. Patients that have expressed high levels of nitric oxide (HNO) have a worse clinic outcome than patients with lower NO levels. This could be due in part to the suspension of the apoptosis pathway by the AIF gene. Furthermore, the IκBα gene has been found within the apoptosis pathway as well. This serves to regulate the NF-κB gene, which allows the formation of anti-oxidants. The activation of this gene could also serve to propagate the replication of cells expressing high nitric oxide. To better understand the effects of NO, our laboratory studied a cell line system of both HNO-adapted and parent cell lines via a gene chip analysis.

Methods: Five parent/HNO pairs (SCC016, SCC040, SCC056, SCC114, and SCC116) of squamous cell carcinoma cell lines, four pairs of parent/HNO pairs (MCF7, Hs578t, T47D, BT20) of human breast adenocarcinoma cell lines, and one pair (A549) of human lung adenocarcinoma cell lines were tested in a gene chip experiment. The gene chip experiment involved dying cell lines green, the HNO cell lines, or red, the parent cell lines, within a microarray plate. The cell lines were then overlayed to determine the magnitude of upregulation (green) or downregulation (red) of all genes in the human genome in these tumor cells.

Results: It was discovered that the Apoptosis Inducing Factor (AIF) gene was commonly down-regulated in all the squamous cell carcinoma cell lines (SCC016, SCC040, SCC056, SCC114, and SCC116). It was also discovered that the IκBα gene was found to be commonly up-regulated in all the Adenocarcinoma cell lines (MCF7, Hs578t, T47D, BT20, and A549)

Conclusions: These results suggest that squamous carcinoma cells adapted to high levels of nitric oxide will show down regulation of the AIF flavoprotein to prolong the lifespan of the malignant cell, allowing it to cause further damage. These results also suggest that the up-regulation of the IκBα gene will cause the NF-κB gene to be deactivated, preventing the formation of anti-oxidants that would reduce the damaging impact that a high amount of Nitric Oxide will cause.

Funding Source: VA Merit Review

Transmembrane Glycoprotein NMB (GPNMB) Is Overexpressed in Head & Neck (H&N) Squamous Cell Carcinomas (SCC) Exposed To Increased Levels of Nitric Oxide (NO)

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Hypothesis: Exposing SCC cell lines to increasing levels of NO can result in the increased expression of genes that contribute to the progression of metastasis.

Objective: Cancer metastasis is a complex process involving a number of highly regulated steps, such as, cell invasion and proliferation. Recent studies have shown that exposure to Nitric Oxide (NO) results in stem cell-like properties of cancer cells. These cancer cells that are exposed to high levels of NO (HNO cancer cells) take on a more aggressive phenotype, resulting in adverse consequences in the progression of cancer. Cancer patients that present with higher levels of NO are observed to have a poor chance of survival when compared to those with lower levels. NO exposed cancer cells have higher metastatic potential when compared to their parent cell lines. In this study, it was found that NO exposed cancer cells overexpress the GPNMB gene, which is believed to have a role in metastasis. GPNMB is a type I transmembrane glycoprotein protein that has been found to be up-regulated in various cancers. A key feature of GPNMB is its tripeptide (Arg-Gly-Asp) RGD motif, which is capable of integrin binding. This activity is important when it comes to the regulation of cell migration, cell adhesion, and other vital processes of metastasis.

Methods: Five H&N cell SCC cell lines were used: SCC016 (tongue), SCC040 (tongue), SCC056 (tongue), SCC114 (floor of mouth), and SCC116 (alveolar ridge). The cell lines were subjected to increased levels of NO by DETA-NONOate until a maximum concentration of 600 mM was reached. RNAs were isolated from these cell lines and their respective parent cell lines. The gene level expression of these NO exposed cell lines were then compared to their individual parent cell lines using DNA microarrays. This data was further compared to a UniProt-GOA association file (Human) by a program, in order to find genes belonging to certain Gene Ontology (GO) terms. The GO term used was GO:0005178, which contains genes related to the molecular function of integrin binding.

Results: It was observed that along with GPNMB being commonly overexpressed in all the five SCC cell lines (SCC016, SCC040, SCC056, SCC114, SCC116) that were exposed to increased levels of NO, they also appeared to be the most consistently up-regulated genes. GPNMB contains an RGD motif in its extracellular domain region, which is recognized by many members of the integrin family. Binding of this ligand motif to integrins can lead to important cell adhesion interactions and other metastatic processes.

Conclusions: Exposure to high levels of NO is correlated with an increase in the expression levels of genes that enhance metastatic potential. In this case, the exposed NO cancer cells were found to overexpress the GPNMB gene. Further study is required to understand the mechanism by which GPNMB contributes to the progression of metastasis. Additional studies with varying concentrations of NO and its effect on GPNMB expression may also be useful in determining the genes significance.

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JUN, FOS, & AP1 Are Upregulated in High Nitric Oxide (NO) Exposed Head & Neck Cancer Cells

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Hypothesis: Exposure and adaptation of Head & Neck (H&N) cancer cell lines will result in up-regulated JUN, FOS & AP1; genes related to increased metastatic potential.

Objectives: Exposure and adaptation to high concentrations of NO causes the H&N tumor cells to express a more aggressive phenotype in comparison to non-exposed cells, which result in greater metastatic potential. Little is known about the process of how these NO exposed cancer cell lines acquire such an aggressive phenotype. In order to mimic what is seen clinically, five H&N cell lines were adapted to high concentrations of NO. AP1 is a transcription factor protein composed of JUN and FOS and is responsible for varying cellular processes including differentiation, proliferation and apoptosis. JUN codes for c-Jun, a proto-oncogene, which has been implicated in aggressive breast and lung cancer cells.

Methods: Five human H&N cells lines (SSC-016, SSC-040, SSC-056, SSC-114, and SSC-116) were studied. The cell lines were exposed to high NO by slow exposure to increasing quantities of DETA-NONOate (NO donor). mRNA isolated from both the parent and NO cell lines were tagged with red/green fluorescent markers. A gene chip analysis was used to assess genome wide gene expression. Additionally, Cell migration rates were assessed via scratch assays. In all five NO exposed cancer cells lines the following genes were found to be up-regulated: JUN, FOS, & AP1. These three genes also demonstrated increased cell migration velocities.

Results: JUN, FOS and AP1 were consistently up-regulated in all the cell lines. Increased migration velocities were demonstrated compared to the parent cell lines.

Conclusions: Exposure to increasing levels of NO results in an up-regulation of JUN, FOS, and AP1 in human H&N cell lines (SSC-016, SSC-040, SSC-056, SSC-114, and SSC-116). Increased migration velocities were demonstrated for JUN, FOS, and AP1 in all cell lines- indicative of tumor aggressiveness.

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Down Regulation of Condensin Complex I Protein in Head and Neck (H&N) Squamous Cell Cancer (SCC) Cell Lines Adapted To High Levels of Nitric Oxide (NO)

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Hypothesis: Down regulation of Condensin complex I gene, NCAPD2, promotes carcinogenesis in head and neck epithelial cell lines.

Objective: One of the many causes of carcinogenesis is due to abnormal changes or functioning in genes responsible for appropriate cell cycle regulation. NO is a free radical by-product produced by all cells of the human body. It helps to communicate and transmit signals throughout the body. Cell lines that are exposed and have accordingly adapted to high levels of NO results in the down regulation of many important mitotic regulator genes involved in cell cycle regulation, thereby leading to chromosomal instability (CIN). NCAPD2 is one such gene that encodes for Condensing Complex I, which plays a crucial role in proper condensation, and segregation of chromosomes in addition to supporting genome stability, cell differentiation and development. To understand the relationship between increased NO levels and any metastatic potential, five H&N SCC cell lines were subjected to high levels of NO. After adapting to the high NO levels, the NCAPD2 gene was observed to be down regulated.

Method: Five pairs of H&N cell lines (parent and cancer cell lines) were studied in this experiment: SCC016, SCC040, SCC056, SCC114, SCC116. Using DAVID Bioinformatics Resources and provided data, cell lines were studied for the genes causing any types of defect in cell cycle processes. It was found that the cell cycle process is interrupted by down regulation of many genes among head and neck cell lines. These genes were separated, and furthermore the collected down regulated genes were compared and analyzed using Blue J, a comparative gene program.

Results: After comparing all down regulated genes, NCAPD2 was the one common gene correlated with cell cycle processes in head and neck cell lines possibly promoting tumorigenesis.

Conclusion: Exposure and adaptation of H&N SCC cell lines to high levels of NO results in the decreased expression of NCAPD2, which is an important mitotic regulator gene involved in cell differentiation and development. This down regulation leads to CIN, which in-turn leads to an observable correlation between high levels of NO and its effect on aggressive or metastatic cancer cell lines. Further studies involving varying levels of NO and its effects on other such similar genes will warrant a better understanding on the subject matter.

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Evaluation of Energy Metabolism Genes in Peripheral Blood Cells of Breast Cancer Patients and Their Prognostic Use

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Hypothesis: Breast neoplasias are the leading cause of cancer mortality among women. Tumor cells are known to be extremely glycolytic, produce excess lactate and proliferate in hypoxic conditions, resulting in metabolic acidosis, regulated by a family of membrane proteins known as monocarboxylate transporters (MCTs). Today, MCT1 and MCT4, MCT family members regulated by CD147, figure on the list of metabolic targets for anticancer therapies.

Objectives: This study aimed to evaluate the potential prognostic value of MCT1, MCT4 and CD147 expressions by qPCR in tumor and peripheral blood samples of under treatment patients with breast cancer.

Methods: Gene expressions of the proposed markers were analyzed by qPCR in tumor and peripheral blood samples of 125 patients and 25 healthy women.

Result: The studied markers were more expressed in tumors with a higher histological grade, demonstrating their relation to tumor development and progression; blood samples collected at diagnosis had increased expression of the markers in relation to healthy women, opening the possibility of using these markers for prognosis evaluations; moreover, in patients with disease progression the expression of these markers is even higher than in those without progression, so that these markers could be used in prognosis evaluation; CD147 expression level was not altered in patients with disease progression after chemotherapy, being a putative chemotherapeutic response marker in liquid biopsies.

Conclusions: After validation, the markers here studied could be a promising strategy in routine laboratory evaluations as breast cancer diagnosis and prognosis.

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Human Papillomavirus, an Oncogenic SUMO Wrestler

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Hypothesis: Oncogenic viruses such as high risk Human Papillomavirus (HPV) often modulate the host cellular machineries involved in various post-translational modifications, for instance SUMOylation, and thereby instigate virus mediated cellular transformation.

Objectives: Viruses are known to utilize cellular pathways to facilitate their survival and propagation. Emerging evidences suggest that SUMOylation pathways are hugely exploited to support viral replication, assembly and evasion of host defense mechanisms. Hence, a detailed understanding of these pathogenic strategies will be extremely helpful in rational design of antiviral drugs that target viruses by preventing their successful hijacking of cellular pathways. To this end, we are investigating the molecular mechanisms involved in smart manipulation of host SUMOylation machinery by HPV.

Methods: Cervical cancer cells C33A, HeLa, Caski and SiHa were used for overexpression and SUMOylation studies. 293T cells were used for Immunoprecipitation experiments. Colony formation assay and Migration assays are described in Chand et al., Carcinogenesis, 2014, 35(8),1830. Transfections were performed using Lipofectamine 2000 (Invitrogen).

Results: Our findings illuminate for the first time that HPV16E6 induces cellular transformation by stimulating degradation of human co-activator protein hADA3 via its enhanced SUMOylation. In another study, we also demonstrate a novel insight into HPV linked oncogenesis by showing the involvement of HPV16 E7 in modulating SUMOylation of the oncogenic factor FoxM1b by impairing its interaction with Ubc9.

Conclusion: These results together exhibit how HPV oncoproteins attack the cellular SUMO machinery and win the battle by manipulating the key enzymes. Such important acumens on SUMOylation as a novel mechanism related to HPV pathogenesis may aid in devising highly selective and more efficient strategies for anticancer and antiviral therapies.

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A Kind of Tumor Therapy and Thinking

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Hypothesis: Sick people most eager to alleviate the symptoms of pain relief. I have a long-term oral treatment medicine capsules their various pathological conditions, can also feel, exploration forward, found what treatment. I found a case of breast tumor cases, assuming this medicine capsules orally to treat tumors.

Objectives: Cancer Research and Treatment. Treatment of this patients
with right chest subcutaneous tumor patients, study the causes of this tumor before and after treatment concept.

Methods: Grind a series of traditional Chinese medicine decoction pieces into powder and put the powder into capsules, daily oral administration of 1 or 2 times, long-term use, and treatment of this tumor-related symptoms. This set includes a variety of traditional Chinese medicine Pieces, in this set, the constantly changing pharmaceutical composition. Ingredients for nourishing yin take up a large percentage in the whole amount of the therapeutic medicine.

Result: The lump was significantly reduced nearly disappeared, the pain disappeared. From the original 14*7*5mm reduced to 2*2*2mm (reduced from the original date pit large as a small grain of rice), a significant treatment effect. Now mass is maintained at a small grain size, no pain.

Conclusions: A series of capsules made of traditional Chinese medicine can treat a tumor and meanwhile many kinds of internal and surgical symptoms, which can make people more comfortable. Ingredients for nourishing yin take up a large percentage in the whole amount of the therapeutic medicine, so this kind of tumor is formed in an environment of internal heat due to yin deficiency. It is correct to use more ingredients that nourish yin. Keeping the body and mind in a comfortable condition is the standard. Therefore, during the treatment, symptoms that are obvious get treated, that is, treating the tumor is not the only task. It seems that this method can treat many kinds of tumor, and other diseases.

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Dichloromethane Extract of *Basella alba* Caused Antireplicative and Antiproliferative Effects in Mcf-7 and Mda-Mb-231 Breast Cancer Cell Lines

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Hypothesis: Breast Cancer is the most common cancer among American women and the second leading cause of death among women. Several factors have been implicated in its incidence. Due to the heterogeneity of breast cancer, several attempts are in place to assess new drugs especially from plant origin for its modulation.

Objectives: In this study, the modulatory effect of dichloromethane extract Basella *alba* on MCF-7 and MDA-MB-231 breast cancer cell lines was assessed.

Methods: *Basella alba* leaves were obtained from Oke Baale Market, Osogbo, Nigeria and authenticated at the Department of Botany, Univeristy of Ibadan (UIH-22501), air-dried and pulverized. Soxhlet extraction of the pulverized powder was done using dichloromethane, the extract was there after concentrated using rotary evaporator. The antiproliferative effect of 200µg/ml and 400µg/ml Dichloromethane Leaf Extract of Ba (DLEBa) was studied in MDA-MB-231 and MCF-7 cells. Reactive Oxygen Species (ROS) production was done spectrophotometrically. Apoptotic induction and cell cycle analyses were determined using flow cytometer while cell viability was by MTT assay. Data were analysed using ANOVA at $\alpha_{0.05}$.

Result: DLEBa caused significant antiproliferative effect, ROS production, and cell cycle disruption in both. MCF-7 and MDA-MB-231 breast cancer cell lines.

Conclusions: The dichloromethane leaf extract of *Basella alba* (DLEBa) produced antiproliferative and antireplicative effects the tested breast cancer cell lines.

Funding Source: N/A

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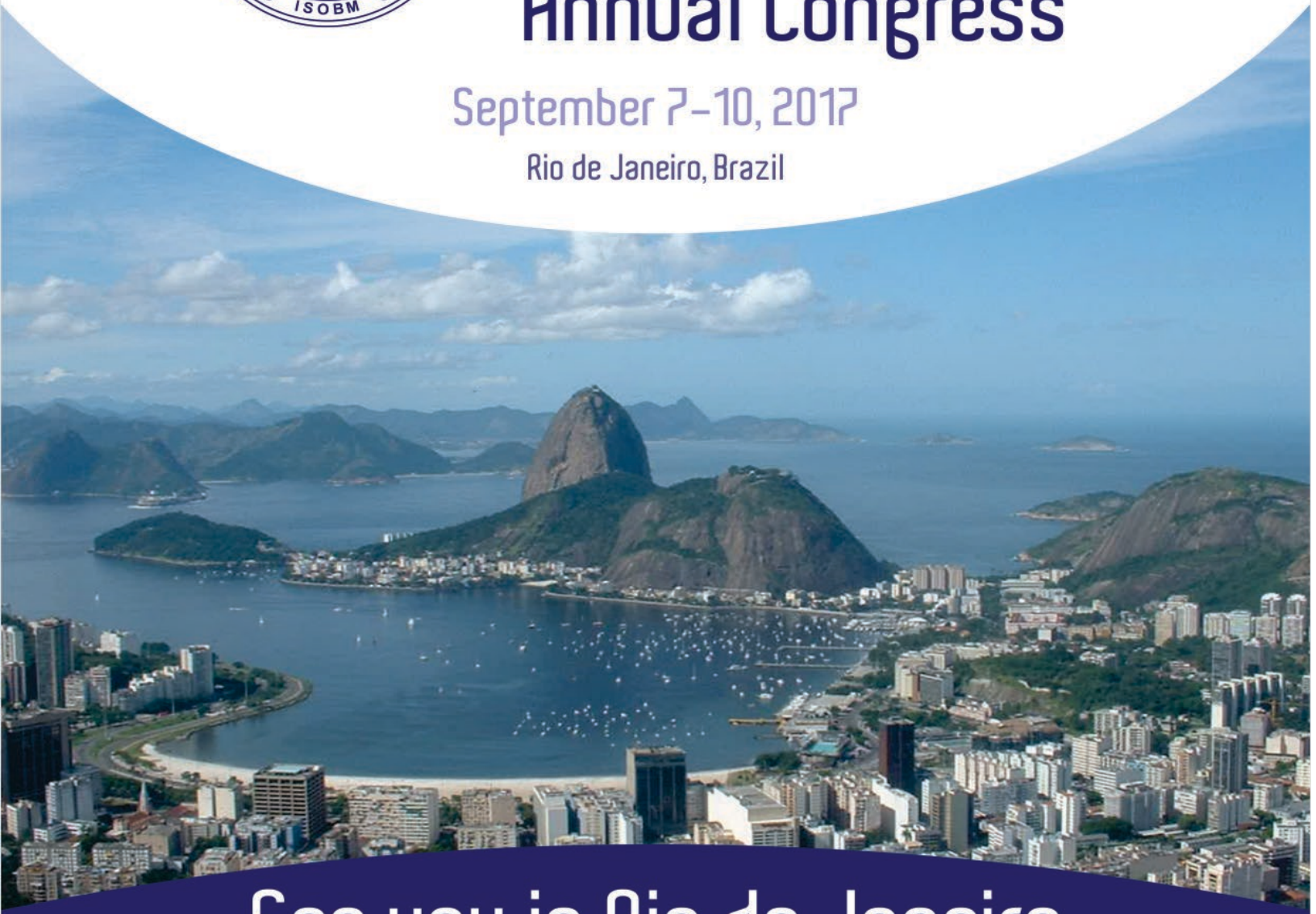

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