Abstracts for publication

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The volatile fingerprint of melanoma

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Background: Melanoma is the most serious form of skin cancer and early diagnosis is the best strategy to combat it. The purpose of this study was to identify volatile and semi-volatile compounds released from melanoma tissue in order to find biomarkers of melanoma. Our hypothesis was that the different metabolic status of nevi and melanoma tissue alters the profile of volatile substances.
Materials and Methods: This was a case controlled study on two groups: biopsies from patients with previously diagnosed melanoma (all histotypes and all stages of melanoma are included) and healthy volunteers. The whole procedure was accomplished according to the approved IRB protocol from University of Miami (No. 20061117) and from Miami Veteran Affairs Medical Center (No. 00762). We collected volatiles from both control (nevi) and melanoma biopsies using head space solid phase micro-extraction (HS-SPME). We used gas chromatography/mass spectrometry to characterize complex volatile profiles. Based on the mass spectrometry data, the identity of each compound was determined from NIST database (NIST/EPA/NIH Mass spectral library) and the Golm Metabolome Database.
Results: In establishing which volatiles are to be used as potential melanoma biomarkers we use both step-wise linear discriminant and fuzzy logic analysis methods. Our preliminary results revealed a broad range of alkanes, methylated alkanes, and aldehydes in melanoma group.
Conclusion: These results suggest that a differential volatile metabolic profile of melanoma does indeed exist. On-going studies will generate a much larger data set that will enable statistical evaluation of our hypothesis.

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Proteomic changes in colorectal cancer cell lines in response to sorafenib treatment

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Background: Sorafenib is an oral, reversible small molecule multi kinase inhibitor of several tyrosine- and serine-/threonine kinases like BRAF, VEGFR 1/2/3, PDGF-β, RET, flt-3, p38 and c-kit. Based on positive results in preclinical studies, there are numerous ongoing phase I/II trials combining sorafenib with conventional therapies in the treatment of colorectal cancer (CRC). However, the molecular effects of this agent on CRC cells are poorly characterized. The identification of molecular biomarkers could be helpful to determine the probability of response to this multi kinase inhibitor and to monitor therapy. Analyses of changes in proteome profiles in response to sorafenib treatment could lead to a better understanding of involved pathways and reveal responsive biomarkers.
Materials and Methods: Two different CRC cell lines (Caco-2, HRT-18) were treated with various concentrations of sorafenib (range 1.0−15 μM) and dose response curves were obtained by WST-1 cell proliferation assay. Subsequently, differential protein expression analysis between untreated and treated samples (IC50 dose) at time point 48 hours was performed with 2D-DIGE (Difference in Gel Electrophoresis) followed by an identification of the particular proteins of interest by mass spectrometry (ESI-MS).
Results: Differential analysis of protein expression levels elucidated nine concordantly regulated proteins (cut off for average ratio: 1.6) in both cell lines under treatment with sorafenib. According to the mass spectrometry results downregulation of the following eight proteins was identified: GART, Septin11, SET, T52DL2, CALM2, S100A9, RPP2, RPS20.
In contrast, the protein ENO1 was found to be upregulated under treatment.
Conclusion: Our results show that proteome-based technologies are a suitable tool for improved understanding of the complex molecular response of malignant cells to treatment with anticancer agents such as tyrosine kinase inhibitors. In response to sorafenib we identified nine regulated proteins that have not yet been associated with this agent. All these proteins have known involvement in cancer-related pathways. Their potential as responsive biomarkers has to be clarified in further functional studies.

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Aurora kinase B: expression and potential therapeutic target in malignant pleural mesothelioma. An in vitro study

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Background: Aurora kinase B (AURKB) belongs to a family of three kinases (A, B and C), which are involved in concert in multiple roles during mitotic progression. Overexpression of AURKB is associated with aneuploidy, aggressive behaviour and poor prognosis in a wide variety of tumour types. AURKB interacts with survivin in driving several mitotic events. AURKB is also required for histone H3 phosphorylation, linked with mitotic chromatin condensation, and cooperates with p16(INK4a) protein in the maintenance of centrosome duplication. More aggressive malignant pleural mesotheliomas (MPM) show a high AURKB expression and for this reason AURKB is of potential clinical interest in the treatment of this fatal cancer following the recent development of small-molecule AURK inhibitors with antitumour activity. AZD1152 is an AURK kinase inhibitor currently in clinical trials and is 1000-fold more selective for AURKB than for AURKA. The aim of this study was to assess the level of expression of AURKB and a few of its molecular partners in MPM cell lines and to determine the effects of AZD1152 on these cell lines.
Materials and Methods: 10 MPM cell lines were used. They had been previously established from pleural effusion fluids drained from patients with histologically proved MPM. Immunocytochemical analysis was performed using antibodies purchased from Cell Signaling Technology, Inc. The effects of AZD1152 on MPM cell growth were analysed using the MTT assay.
Results: By immunocytochemical staining, basal expression of AURKB and related proteins was evaluated in the 10 MPM cell lines. AURKB, survivin and phospho-histone H3 showed an intense nuclear expression in all MPM cell lines: almost 100% of cells expressed AURKB and survivin, whereas phospho-histone H3 expression was limited to 5−10% of cells. On the other hand, p16 expression was weak and cytoplasmatic and the proportion of p16-positive cells differed from one MPM cell line to another. Four MPM cell lines were treated with AZD1152 (gift of Astra Zeneca), which induced apoptotic cell death.
Conclusion: These finding constitute the rationale to further investigate the AURKB pathway in MPM and in addition they may be important in the selection of patients to benefit from clinical trials of AURK inhibitors.
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Transcribed ultraconserved elements expression in tumour cell lines

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Background: Ultraconserved regions (UCRs) are a set of conserved sequences between humans, mice and rats, longer than 200bp. Most of these UCRs are transcribed (T-UCRs) and may be involved in regulatory functions forming part of the large cohort of non-coding RNAs. Proof of their regulatory role is that T-UCR expression and sequence is altered in several cancers, and many T-UCRs have been mapped into cancer-associated genome regions, a number of fragile sites and genomic regions affected in several tumours. We think that T-UCRs, like microRNAs, may be expelled to blood in disease states. Taking into account these data, we think in searching a number of T-UCRs with constant expression in normal tissues which could be used to assessing alterations in the amount of total T-UCRs in plasma and/or serum from patients with cancer.
Materials and Methods: We look for T-UCRs with stable expression and suitable characteristics into available databases to finding candidate T-UCRs. Initially, the expression of these T-UCRs was assessed in colon, breast, and melanoma tumour cell lines. For these analyses, we use qRT-PCR with validated primers for selected T-UCRs (Primer Design Co., Ltd.).