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.
Grant support: Regione Piemonte − Bando Regionale 2008 per il Finanziamento di Progetti di Ricerca Sanitaria Finalizzata and the Alessandria branch of the Italian League against Cancer.

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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.).

As housekeeping we use 18S (Primer Design Co., Ltd.), such as was previously depicted.
Results: We have selected for this study three T-UCRs: uc.277, uc.301, and uc.440. We have found that the amount of these T-UCRs is generally low. T-UCR uc.440 was found over-expressed (nearly 16 times) in MDAMB231 breast cancer cell line versus healthy tissue ($p < 0.01$). T-UCR uc.277 is also slightly over-expressed in MDAMB231 ($p < 0.05$). In addition, T-UCR uc.440 was found slightly over-expressed in HT29 colon cancer cell line regarding to the others analyzed colon cancer cell lines ($p < 0.05$), but there weren't found differences regarding to healthy tissue. It could not be detected significant expression of the T-UCR uc.301 in any tumour cell line. In melanoma cell lines could not be detected the expression of any assayed T-UCRs.
Conclusion: Our goal was to find a T-UCR with a constant and high expression in tumour cell lines regarding to healthy controls. We have found that T-UCR uc.440 is significantly upregulated in MDAMB231 breast cancer cell line regarding to healthy control, but also regarding to the others breast cancer cell lines analyzed. No relevant changes in the expression for the others T-UCRs tested were observed. For our knowledge, this is the first study where T-UCRs are assayed in colon, breast and melanoma tumour cell lines.

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Rho C in melanoma: possible target for statin treatment?

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Background: There is large interest in statins as agents in cancer prevention and treatment. In more than 1300 melanoma cases statin use was associated with reduced Breslow thickness. Gene expression profiling experiments in melanoma cell lines revealed RhoC to be upregulated in cell lines with high metastatic capacity. We demonstrated that RhoC immunohistochemical expression in primary cutaneous melanoma was strongly associated with thicker and ulcerated tumors. Specific inhibition of Rho C in the A375M melanoma cell line reversed migration and invasion. Statins can block activation of RhoC by blocking geranylgeranyl pyrophosphate and farnesyl pyrophosphate which are important for isoprenylation of Rho proteins. Atorvastatin reverted the metastatic phenotype of several human melanoma cell lines and inhibited in vivo metastasis in SCID mice injected with A375M melanoma cells.
We explored if the statin simvastatin can influence the growth rate of melanoma cells of the BLM melanoma cell line.
Materials and Methods: The effect of different concentrations simvastatin (50, 500 and 5000 nM) on the global growth rate of BLM melanoma cell lines was investigated and compared to untreated BLM cells (control). By using 12 semi-automatic phase-contrast microscopes the relative increase of BLM cells was measured at 24, 48 and 72 hours.
Results: The simvastatin 5000 nM concentration significantly reduced global growth rate of the BLM cells after 48 and 72 hours ($p < 0.001$) with stabilization of the cell population. There were no significant differences in global growth rate of the 50 nM and 500 nM simvastatin groups compared to control.
Conclusion: These observations support a growth inhibitory effect of high concentrations of simvastatin on the BLM melanoma cell line. Further research will focus on the determination of growth inhibitory concentrations of different statins on different melanoma cell lines.

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Detection of circulating tumor cells (CTCs) in Stage cT3−4 or N+ rectal cancer patients (pts) undergoing combined neoadjuvant therapy plus curative surgery. Preliminary data

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Background: CTCs detected at baseline and at disease-evaluation time-point during treatment seem to be an independent prognostic factor in metastatic colorectal cancer (Cohen SJ JCO, 2008). CTCs' role as predictive marker in early stages after radical surgery is under investigation, while no data are available in locally advanced rectal cancer suitable for neoadjuvant chemoradiotherapy. Aim of the study is to investigate the role of CTCs in local advanced rectal cancer pts undergoing neo-adjuvant chemo-radiotherapy (CT-RT).
Materials and Methods: In a prospective single institution study, cT3−4 or N+ rectal cancer pts staged by transrectal ultrasound and/or pelvic MRI and chest-abdomen CT scan, are submitted to capecitabine (825 mg/mq, orally, twice daily continuous) with concomitant radiotherapy (50.4 Gy/fractions to the primary tumor and perirectal nodes), followed by two cycles of capecitabine (1250 mg/mq, orally, tid 14/21 days). Primary endpoint is evaluation of CTCs at baseline (t0), after neoadjuvant therapy, before surgery (t1), after surgery (t2), and at 6-month follow-up (t3) and its correlation with survival parameters. CTCs are enumerated with immunomagnetic separation in 7.5 ml peripheral blood at over-mentioned time-points (CellSearch System, Veridex Inc).
Results: Twenty-six pts (16M; 10F; median age:63$\pm$13 yrs; range: 44–83 yrs) underwent t0 sampling, 8 pts completed CT/RT and therefore underwent t1 and t2 sampling. At baseline (t0) three pts presented 1 CTC (12%), one 2 CTCs (3.5%), one 27 CTCs (3.5%) while in twenty-one (81%) no CTCs were detected. At t1 and t2 none of the eight pts analyzed showed CTCs.
Conclusion: CTCs $\geqslant$1 are present in 15% of our patients, but the sample is too small for statistical analysis. The study is still ongoing; more data will allow to assess prognostic and predictive significance of CTCs during treatment in this setting.

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Assessment of oxidative stress in tumor cells and normal mucosa cells from head and neck squamous cell carcinoma patients

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Background: One of the cancers particularly linked to oxidative damage and oxidative stress is the head and neck squamous cell carcinoma (HNSCC). Tobacco and alcohol are well defined etiological factors.
Concurrent radiation and chemotherapy, a recognized alternative treatment to surgery for patients with advanced HNSCC, can induce a systemic oxidative stress. Oxidative damage is the main mechanism mediating the clinical effect of radiotherapy, and an increased resistance to oxidative stress by malignant cells is associated with treatment failure. Response to (chemo) radiation treatment varies from patient to patient.
The purpose of this study was to compare the tissue levels of glutathione in HNSCC tumoral tissue (Tum) and adjacent normal mucosa (Muc) biopsies as a potential factor of variability in (chemo) radiosensitivity.
Materials and Methods: 27 newly diagnosed HNSCC patients were prospectively studied. All were current smokers. 27 tumoral biopsies and an equal number of biopsies from normal mucosa were analysed. The oxidised/reduced glutathione ratio was measured with the capillary electrophoresis Ceofix GSH/GSSG kit (Analis, Namur, Belgium). Two hundred µl of whole blood, normal and tumoral tissues were immediately grinded with 600 µl of 5% metaphosphoric acid. After centrifugation (within 3 hours), 100 µl of the supernatant was mixed with 400 µl of the kit diluent containing naphthalene sulfonic acid as an internal standard. Analysis was done on a P/ACE 5000 series with a 37 cm and 75 µm i.d. capillary maintained at 25ºC. The separation was realized at 8 kV with a pH 8.2 borate buffer containing SDS. The glutathione peaks were detected at 200 nm and integrated as under-the-curve areas (AUC). The results are expressed as the ratio of oxidised GSSG AUC to the reduced GSH AUC. Clinico-pathological parameters were also considered as potential factors of variability in oxidative stress status of HNSCC tumoral tissue.
Results: The GSSG to GSH ratio was higher in the tumoral tissue than in the adjacent normal tissue in 12/27 of the cases.
Conclusion: In 44% of our cases, HNSCC tumoral tissue from untreated patients had a GSSG/GSH ratio different than that found in the normal adjacent cancer free mucosa. This difference was not related with clinico-pathological parameters. Heterogeneity in HNSC cancer is emphasized. The ability of the GSSG/GSH ratio to predict differential chemoradiosensitivity will be evaluated by long-term survival data.

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Reduced p63 expression in myoepithelium correlating with increased invasiveness in epithelium

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Background: Our previous studies revealed that pregnancy associated breast cancer (PABC) had significantly reduced nuclear p63 expression