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Novel proteins highly expressed in tumors identified by a high throughput immunoproteomic approach

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Cancer biomarker discovery is an extremely active research field in both academia and pharmaceutical companies. Several high throughput technologies were applied through the years to identify proteins specifically related to cancerous phenotype. However, most putative biomarkers identified by conventional proteomic/transcriptomic strategies still need to be clinically validated. Here we present a cancer biomarker discovery approach, based on the use of a large library of antibodies raised against recombinant human proteins, to detect, by immunohistochemistry analysis of excised tumor samples, tumor-associated proteins. Starting from the whole human genome, genes encoding proteins predicted as membrane or secreted were selected and then cloned and expressed by high throughput techniques. Recombinant proteins were used to build a polyclonal antibody library (YOMICS®) currently comprising more than 1,700 murine immune sera. The ability of sera to recognize specific targets predominantly present in tumors was assessed by Tissue Micro Array (TMA, a miniaturized immunohistochemistry analysis) performed on tumor tissue samples and healthy controls from pedigreed patients affected by the most common human tumors (including colon, ovary, breast, lung, and prostate cancers). Even if the screening is still in progress, eight antibodies were identified showing high reactivity on a high percentage (ranging from 40 to 95%) of tumor tissues concerning to one or more cancer types, showing negative staining on correspondent normal tissues. The corresponding protein targets, being novel tumor-associated proteins, were validated and then characterized both at cellular and molecular level by analyzing their expression, cellular localization and biological role in a panel of tumor cell lines. Interestingly, three of the above-mentioned proteins are localized at the plasma membrane, while the other ones are intracellular. Furthermore data so far available show that three proteins are involved in cellular processes relevant to tumor development. A reduced expression of EXN4 and EXN7 impairs the invasive phenotype of considered tumor cell lines, while over-expression of EXN1 increases the cell clonogenic phenotype. Finally the newly identified proteins are promising candidates as new tumor biomarkers and could be exploited to develop target-specific drug therapies.

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Soluble Axl (sAxl) from serum defines a subpopulation of patients with breast, colon, lung, and pancreatic cancers

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Background: Axl is a receptor tyrosine kinase involved in cell growth, differentiation, and migration. It is overexpressed in multiple tumor types and has been implicated in cancer progression and metastasis. The extracellular portion of Axl is characterized by immunoglobulin-like and fibronectin type III repeats, in addition to a binding site for its natural ligand, Gas6. Binding of Gas6 results in the autophosphorylation of Axl and in the activation of multiple cytoplasmic substrates, including members of the PI3K/AKT pathway. Additionally, the extracellular portion of Axl undergoes processing by ADAM10 to yield a soluble form of the receptor. It is postulated that this proteolytic cleavage event is a negative feedback mechanism to downregulate activated AXL and to capture free circulating Gas6.
Materials and Methods: An ELISA system was used to examine sAxl levels in conditioned media from cell lines and in serum samples from patients with colon, lung, breast or pancreatic cancer (drawn prior to chemotherapy). Western blotting and realtime PCR were used to determine Axl expression in cell lines.
Results: The average levels of sAxl in the serum were found to be 27.2 ng/mL (n = 23) in healthy control specimens, and for cancer patients, 35.8 ng/mL (n = 10; p = 0.03) in breast, 35.9 ng/mL(n = 10; p = 0.059) in colon, 40.5 ng/mL (n = 10; p = 0.086) in lung, and 43.1 ng/mL (n = 16; p = 0.012) in pancreas. sAxl levels in tumors averaged at least 31% higher than the sAxl levels in healthy control specimens. Interestingly, in each tumor-specific group there were several (approx. 1 in 4) specimens with extremely high levels of sAxl. Furthermore, sAxl in the conditioned media from pancreatic cell lines was differentially expressed across the panel, and showed good correlation to expression of the Axl receptor at the RNA and protein levels in these cell lines.
Conclusions: We demonstrate that sAxl can be readily detected in cell culture media and in human serum. sAxl levels strongly correlate with Axl kinase expression and sAxl levels are elevated in some cancer patients. Efforts are currently underway to better understand correlations between sAxl expression and other parameters, such as treatment response, overall survival, and histological differences in these patients.

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Looking for a stemness signature in prostate cancer

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Background: Recent data suggest that tumor initiation and growth might be driven by a rare population of cells endowed with stem-properties, and therefore defined as Cancer Stem Cells or Cancer-initiating Cells (CIC). This model might explain the inefficiency of specific anti-cancerous therapies, most of them targeting the bulk of the tumor and not specifically these cancer-initiating cells. CIC may have properties similar to those of normal stem cells, such as self-renewal and differentiation potential and could also share some molecular pathways with embryonic pluripotent stem cells. In this study, we aimed at evaluating the expression of pluripotency and stemness-associated genes in PCA cell lines and prostatic tissues, including PCA and Benign Prostatic Hyperplasia (BPH).
Material and Methods: The study was performed on PC3, Du145 and LNCaP PCA cell lines and on prostate clinical specimens, including PCA and BPH. Expression of specific genes, such as CD133, Oct4, Nanog, Sox2, Klf4, c-Myc, and Chromogranin A (CgA) was evaluated by quantitative real-time PCR. Protein expression was assessed by FACS analysis, or immunofluorescence. Additionally, tumor samples were collected and used for a tissue microarray (TMA), assessing the expression of CgA and Klf4.
Results: PCA cell lines were found to express the specific pluripotency related factors, Oct4, Nanog, Sox2, Klf4, and c-Myc, highlighting the possible presence of pluripotent cells within tumorigenic prostate cell lines. Moreover, the same genes were also well expressed in almost all clinical samples investigated. Notably, there was a trend toward an increased expression of all pluripotency-related genes in tissues from patients bearing PCA, as compared to BPH tissues. In particular, c-Myc and Klf4 genes were significantly more expressed in cancer tissues as compared to BPH and showed a close relationship in their expression as well as with the other pluripotency-related factors. Moreover, analysis of the TMA revealed that Klf4 protein was specifically localized in rare scattered cells within the basal compartment, morphologically similar to neuroendocrine cells. Supporting these results, a high correlation was found between Klf4 and CgA expression.
Conclusions: Taken together, these results indicated the presence of a specific stemness signature in prostatic tissues. In particular, Klf4 gene was significantly more expressed in cancerous tissues as compared to BPH samples. Additionally, Klf4 protein appeared to be expressed by a sub-population of neuroendocrine cells within the prostate, highlighting the potential importance of these cells in prostate cancer progression.

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Level of c-kit expression on pre therapeutic mediastinal lymph node biopsy does not predict its level of expression on post chemotherapy lung tumor

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Background: Experimental evidence suggests that stem cell factor (SCF) and its receptor c-kit (CD117) play an important role in survival and proliferation of lung cancer stem cells (CSC), providing a promising target to neoadjuvant treatment of non-small cell lung cancer (NSCLC). However, efficacy of c-kit inhibitors relies on c-kit expression on the primary tumor, and some patients with N2 NSCLC are diagnosed on the basis of mediastinal lymph node biopsy. Therefore, we sought to determine whether the level of c-kit expression on pre therapeutic mediastinal lymph node biopsy predicts its level on lung primary tumor.