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Poster Session
Translational Research

118 Up-regulated proteins in the fluid bathing the tumour cell microenvironment as potential serological markers for early detection of cancer of the breast

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Background: Currently, there is an urgent need to identify biomarkers that can facilitate the early diagnosis of breast cancer as the disease is easier to treat and cure if it is detected early at a localized stage. Moreover, clinically useful biomarkers are expected to lead to a significant reduction in mortality. Here we describe a detailed gel-based proteomics analysis of the tumour (TIF) and normal interstitial fluids (NIF) collected from 69 prospective breast cancer patients with the aim of identifying abundant cancer up-regulated proteins that are common to most breast cancers and that may represent potential serological markers for the early detection of breast cancer.
Materials and Methods: TIFs and NIFs were recovered within 30–45 min from the time of surgical excision as previously described [1]. Samples were analyzed by 2D PAGE coupled with MALDI-TOF MS/MS and validation of the markers was performed by immunohistochemistry in an independent set of patients using tissue microarrays.
Results. A systematic computer assisted analysis of the 2D gels of 69 TIF samples and controls revealed a set of 26 breast cancer markers that were up-regulated in 90% or more of all the TIFs. Most of these proteins were also identified in a carefully selected TIF sample using LC-MS/MS. The expression of calreticulin, cellular retinoic acid-binding protein II, chloride intracellular channel protein 1, EF-1-beta, galectin 1, peroxiredoxin 2, platelet-derived endothelial cell growth factor, protein disulfide isomerase and ubiquitin carboxyl-terminal hydrolase 5 was further validated using a tissue microarray containing 70 malignant breast carcinomas of various grades of atypia.
Conclusions: The set of 26 breast cancer markers revealed in this study represent potential serological markers for the early detection of breast cancer as a significant number of these proteins have already been detected in the blood/plasma by others. The next step, which will require marker prioritisation, will have to take into consideration expression by other tissues and tumours, detection in the blood using independent assays, as well as availability of specific antibodies.

Reference(s)

[1] Gromov P, Gromova I, Bunkenborg J, Cabezon T, Moreira JM, Timmermans-Wielenga V, Roepstorff P, Rank F, Celis JE. 2010. Up-regulated proteins in the fluid bathing the tumour cell microenvironment as potential serological markers for early detection of cancer of the breast. Mol Oncol 4, 65–89.

119 The E3-Ubiquitine ligase c-Cbl protects cells against oxydative stress – usefulness as a prognostic marker and a possible therapeutic target

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Background: The c-Cbl ($p120^{cbl}$) E3-Ubiquitine ligase is directed against several tyrosine-kinase growth factor receptors and functions as a down regulator of these activated receptors. This protein is also considered as a molecular poly-adaptor susceptible to specifically link to an increasing number of signalling proteins. The combination of these two functions would allow c-Cbl to participate in the internalization of the targeted receptors and activate down-stream effectors. Several works including ours have showed its involvement in apoptosis and transformation, and we report new related leads about c-Cbl, apoptosis and malignancy.
Material and Methods: p120 c-$cbl^{-/-}$ (KO) or c-$cbl^{+/+}$ (KO) mice, treated or not with flutamide and/or castration, were explored for c-Cbl apoptotic androgen-dependent modulation. The apoptotic signalling pathway was assessed for protein or/and mRNA expression and number of apoptotic cells (TUNEL, DAPI experiments). Primary mouse embryonic fibroblasts (MEFs) from c-Cbl KO or WT mice as well as prostate cell line LNCaP were assessed for expression of c-Cbl upon etoposide or hydrogen peroxyde apoptotic conditions. Interference with c-Cbl mRNA was performed for ROS expression in LNCaP. Human carcinoma and sarcoma from diverse origin, benign prostatic hypertrophia, compared to healthy tissues were assessed for c-Cbl expression through western blots or Tissue Microarrays (TMA) *in situ* studies.
Results: We showed through *in vivo* and *in vitro* experiments that c-Cbl functions as an anti-apoptotic regulator in prostate, MEFs or LNCaP. Our studies of KO versus WT MEFs clearly showed that c-Cbl is highly susceptible essentially to ROS, protecting cells against oxidative stress. We then observed in numerous human tumours a high expression of c-Cbl, often associated to an important oxidative stress (tested by the anti-endonuclease APE1). We confirmed (already reported) c-Cbl over-expression in prostate adenocarcinoma, correlated to cancer gravity. We spray out this observation to other tumours, through the *in situ* staining of numerous TMA spots, showing our observation could be widen to several malignant tissues: ovary, uterus, brain, colon, rectum, lymphoma, striated muscle. Preliminary experiments, supported by the known c-Cbl regulatory role in energy expenditure, strongly suggest that c-Cbl could also participate to ROS generation.
Conclusions: c-Cbl could be used as a malignant marker in cancer prognosis. It could likely protect malignant cell against oxidative stress and be used as a therapeutic target in human cancer (US Patent in progress).

120 NF-kappaB, a crucial regulator of the HIF-1alpha and HIF-2alpha response

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Considering the heterogeneity of metastatic tumours and that the progressive transformation of cells requires a multistep process; logic dictates that cancer is ultimately driven by a combination of several signaling pathways that are interconnected.
NF-κB and HIF are important survival factors that have been implicated in tumourigenesis, cancer progression and metastasis. Both can be activated by similar stimuli such as oncogenes, inflammatory cytokines (e.g. TNF-α or IL-1β), reactive oxygen species (ROS) and hypoxia. In addition, NF-κB and HIF share a number of target genes (e.g. VEGF) and are involved in similar responses such as inflammation, growth and survival. Given the potential for crosstalk, this study explores the mechanistic and functional role of NF-κB in the HIF-mediated responses under hypoxic and normoxic conditions.
Experimental procedures include the use of various cell lines, siRNA studies, overexpression, cytokine treatment, as well as chemical inhibition of targeted proteins. Techniques such as quantitative PCR, western blotting, Chromatin IPs, and prediction software have been utilised to analyse the interactions of these pathways at a molecular level.
We have found that under normoxic conditions, following TNF-α stimulation, NF-κB activates the transcription of the HIF-1α gene, which results in upregulation of the HIF-1α subunit. This correlates with increased HIF-1α activity, recruitment to target genes and upregulation of transcription.
In addition, we have found that HIF-2α is also increased following TNF-α in a NF-κB dependent manner. However, NF-κB does not increase HIF-2α mRNA, but acts as an indirect regulator of HIF-2α protein stabilisation. In this study we show that HIF-2α is stabilized via a mechanism dependent on the presence of HIF-1β (ARNT). Interestingly, our data demonstrate that NF-κB dependent regulation of HIF-1β results invariably in the upregulation of HIF-2α levels as well as HIF-1α following either hypoxia or TNF-α stimulation under normoxic conditions.
Taken together our results indicate that NF-κB maintains a multifacetted regulatory function by controlling more than one HIF pathway component transcriptionally, thereby, as a consequence, exerting significant influence over HIF-α protein levels and activity.
Ultimately, these findings support the notion that key regulators of the inflammatory process (NF-κB and HIF) are likely to have therapeutic implications for many diseases, including cancer as NF-κB and HIF constitute an important mechanistic link between chronic inflammation and solid tumours.