213 Characterization of novel recombinant human single chain cancer-specific antibodies

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Background: Our overall goal is to identify antibodies for possible use in diagnostics and therapy utilizing phage-display technology and cell specific antibody selection.
Material and Methods: A breast cancer cell line, PM-1, and the colorectal cancer (CRC) cell lines HCT-116 and HT-29 were used to screen for breast and CRC specific antibodies, respectively. Three unique recombinant antibodies were screened for binding to a panel of cancer cell lines and normal cells using immuno-magnetic bead selection (IMS) or flow cytometry. Cancer cell specific binding was investigated using immunohistochemistry (IHC) on frozen normal tissue and tumour tissue of different histology.
Results: IMS and flow cytometry analyses revealed differences in binding patterns comparing the three antibodies. CRC-1, one of the anti-CRC antibodies, bound to cancer cell lines of various origins. Endothelial cells were negative for CRC-1 binding. However, binding was observed when testing vascular smooth muscle cells and mononuclear cells (MNC) isolated from normal bone marrow. The other CRC antibody only bound to CRC cell lines and only a fraction of the cells displayed positive binding. The breast cancer cell binding antibody BC-1 bound to most of the cancer cell lines tested, and did not bind to endothelial cells or normal bone marrow MNC.
In IHC positive CRC-1 staining was observed in clinical cancer samples and in normal tissue. Surprisingly, cancer epithelia were not stained, blood vessels stained positive, and myo-epithelial cell staining was observed in breast tissue samples (cancer and normal). Positive BC-1 staining was on the other hand highly cancer specific. The BC-1 antigen is identified as ALCAM and the antibody inhibited cancer cell invasion through Matrigel and reduced tumour growth in nude mice.
Conclusion: We have characterized novel recombinant human single chain antibodies recognizing differentially expressed antigens in cancer cell lines. Additionally, one antigen is expressed also on vascular smooth muscle cells and in normal bone marrow cells, possibly perivascular progenitor cells. The anti-ALCAM antibody is highly cancer specific and inhibits cancer cell invasion and tumour growth.

214 Molecular characterization of patient tumour-derived breast cancer xenografts shows a strong genomic and gene expression stability

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Background: Identifying new therapeutic agents for breast cancer (BC) treatment requires preclinical models that recapitulate the molecular characteristics of their respective clinical tumours. In this work, we analyzed the genomic and expression profiles of human BC xenografts and their corresponding patient's tumours.
Material and Methods: 18 breast cancer xenografts (2 HER2 positive, 6 estrogen receptor positive (ER+), and 10 triple-negative) were obtained by grafting tumour fragments from patients into Swiss nude mice. The molecular characterization of patient's and xenograft's tumours was performed by DNA copy number analysis using a home-made BAC array (aCGH) as well as gene expression profiling using Affymetrix HGU133 Plus 2.0 Microarrays.
Results: We compared the genomic profiles of BC xenografts with those of respective patient's donor tumours by calculating the correlation coefficient based on the status of the probes on each pair of chromosomes. We found that 14/18 paired tumours (78%) had a correlation coefficient higher than 0.50. aCGH based unsupervised hierarchical clustering analysis showed that 16/18 pairs segregated together and revealed the presence of the different molecular classes (ER+, triple negative and HER2+).We next analyzed the gene expression profile of the paired primary tumours and xenografts as described in molecular subtype's classification and found that they showed no or few variations. Interestingly, we identified 558 Probesets corresponding to 371 unique genes differentially expressed in more than 84% of paired tumours. 536 were under-expressed and 22 over-expressed in the xenografts. Immune response, response to wounding, extracellular matrix component, cell adhesion, and angiogenesis constituted the main categories thus identified. An analysis in a public dataset of 1143 breast cancer samples evaluated the prognostic value of the 558 Probe Sets and showed a significant enrichment in genes with prognostic ability. The underexpression of human stromal compartment related genes in the xenografts may highlight its involvement in the prognosis of breast cancer patients. Finally, further analyses on xenografts at different tumour passages did not reveal important changes in the genomic rearrangements, neither in the gene expression profiles. These data suggest a striking genetic stability of the models across the time.
Conclusions: This panel of human BC xenografts accurately maintains the genomic and the expression profiles of their corresponding patient's tumours and are stable across sequential in vivo passages. Consequently, these xenografts represent an ideal model for preclinical investigation of new therapeutic agents. In addition, the lack of human stromal compartment in the xenograft model gave indirectly access to the stromal gene expression profiles of the primary tumour and confirmed its major involvement in the prognosis of breast cancer patients.

215 Study of the regulation of DUSP6 expression as a chemorresistance mechanism in exocrine pancreatic cancer

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Several reasons can explain the poor survival associated to pancreatic cancer: A late diagnosis, a high methastatical potential, and the failure of conventional antineoplasic treatments. We are particularly interested in the search of new therapeutical approaches for the treatment of patients affected with pancreatic cancer. We had previously generated pancreatic cancer cellular models resistant to gemcitabin, antineoplasic drug that has been considered the standard treatment for this type of tumour. IMIM-PC1, IMIM-PC2, and RWP1 pancreatic cancer cells were treated with increasing concentrations of gemcitabin. The RNA from the cells resistant to gemcitabin 500 nM was isolated and, the differential gene expression associated to gemcitabin resistance, was determined by DNA microarrays analysis, comparing gene expression between the parental gemcitabin sensitive cell lines and the resistant cell lines. Several genes were affected in their expression in response to gemcitabin resistance. One of the genes commonly overexpressed in the resistant cell lines is the dual phosphatase DUSP6, enzyme that inactivates the MAP kinases through dephosphorylation in serine and threonine residues. The inhibitory effect of DUSP6 on the MAP kinases-signalling pathways, suggest an important role of this phosphatase in the regulation of a high variety of physiological processes. We analysed in other pancreatic cancer cells (HS-766T, HPAF-II, SKPC-1), the level of expression of DUSP6 and the degree of resistance to gemcitabin, finding a good correlation between both parameters.
We obtained a pancreatic cancer cell line (IMIM-PC2/src) stably transfected with a constitutively active mutant of the tyrosine kinase src; this cell line shows an increased resistance to gemcitabin and high levels of DUSP6 mRNA with respect to the parental untransfected IMIM-PC2 cell line, indicating again a relationship between DUSP6 expression and gemcitabin resistance.
In order to determine the role of DUSP6 in gemcitabin resistance in pancreatic cancer, HS-766T and HPAF-II cells have been transfected with a plasmid that expresses a small interference RNA for DUSP6. We will determine if the decrease in DUSP6 expression correlates with an increase in the sensitivity to gemcitabin in the transfected cell lines. If this is the case, we can consider that the regulation of DUSP6 expression can be considered as a target for the improvement of sensitivity to gemcitabin in pancreatic cancer patients.

216 Selective cytotoxicity of parvovirus H-1 infection for human neuroblastoma and medulloblastoma cells

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Background: In paediatric neuro-oncology, patients with high-risk neuroblastoma and metastasized medulloblastoma both represent subgroups of poor prognosis. Thus, new modalities such as oncolytic virotherapy are urgently required for the treatment of these patients. The rodent parvovirus H-1 (H-1PV) is a non-recombinant wildtype virus apathogenic in animals and in humans. For its efficient cytopathogenicity and full life cycle H-1PV depends on the transformed phenotype of cells. From the aspect of clinical safety, this natural oncoselectivity renders H-1PV a very interesting virus for oncolytic virotherapy in children.
Recently it has been shown that H-1PV selectively kills glioma cells *in vitro* and is oncolytic for gliomas in animal glioma models (Geletneky et. al., 2010). In order to determine its oncolytic potential in paediatric tumours we analyzed cytotoxicity of H-1PV in neuroblastoma and medulloblastoma cells *in vitro*.
Materials and Methods: Neuroblastoma cell lines with different MYCN status (n = 11), medulloblastoma cell lines (n = 7), and normal primary brain cells were infected with H-1PV. We determined efficiency of infection, viral replication, and effects of H1-PV on the cell cycle and cell viability *in vitro*.
Results: Non-neoplastic infant cells (glia cells, astrocytes and neuronal cells in short term culture) were unaffected in viability and morphology after H1-PV infection.