416 Characterization and quantification of macrophages in colorectal cancer by an automated cell system

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Background: Communication between tumour and its surroundings (the tumour microenvironment) is of utmost importance in determining the faith of the tumour. Both pro- and anti-tumour interactions have been described.
In order to understand the role of tumour-associated macrophages in malignant progression we investigated the distribution of total macrophages CD68+ and M2 anti-inflammatory activated macrophages CD163+ in colorectal cancer and adjacent mucosa using a fully automated microscope-based cell analysis system that performs in-situ analysis of multiple markers in complex tissues like tumour specimens.
Material and Methods: Immunofluorescent staining was performed in paraffin sections from 8 G2 colorectal tumours. 4 patients have already developed liver metastasis. Sections from mucosa adjacent to tumour tissue as well as 3 healthy mucosae were examined. Large tissue sections of around 2 cm^2 were scanned using TissueFAXS Cytometer and analysed by TissueQuest analysis software (TissueGnostics GmbH, Austria).
Results: The analysis of the adjacent mucosa revealed a heterogeneous increase in patients regarding local density of CD68+ and CD163+ compared with healthy mucosa. Numbers of CD68+ cells at the tumour front increased twofold in patients without liver metastasis and threefold in patients with liver metastasis. The tumour centre of patients without liver metastasis showed the lowest amount of CD68+ infiltrating cells. Tumour centre of patients with liver metastasis revealed a 4 times increase of CD68+ cell density when compared with patients without liver metastasis. CD163+ cells were present in all tumour areas with high variability, with higher density in patients without liver metastasis.
Of special interest is an observation regarding the presence of mono- or multinucleated CD163−/CD68+ giant cells in tumour and/or adjacent mucosa of all patients without liver metastasis. Only 2 adjacent mucosae from patients with liver metastasis showed macrophages with giant cell phenotype.
Conclusions: Our data suggests that CD68+ giant cells might be a marker for favourable prognosis in colorectal cancer. Further studies are needed to characterize their function.

417 A c-Myc induced gene expression signature in human germinal center B cells predicts subtypes of aggressive non-Hodgkin Lymphoma

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Background: Aggressive Non-Hodgkin Lymphoma (aNHL) are a heterogenous group of malignancies derived from germinal centre B (GC B) cells. Burkitt's lymphoma (BL) is the most homogeneous aNHL entity, characterized by an aberrant expression of the proto-oncogene c-Myc. Recently BL was defined by a specific gene expression signature including c-Myc as one hallmark. Despite an abundant number of cell line investigations and murine models, there is a lack of experimental systems to investigate the role of c-Myc for the transformation of human GC B cells. Therefore we expressed c-Myc in primary tonsillar GC B-cells and monitored expression changes using microarray gene expression profiling. We performed analyses integrating expression profiles from clinical lymphoma samples pointing us to potential mechanisms of disease initiation and progression. Furthermore we asked whether these changes permit to further subgroup aNHL.
Materials and Methods: Purified human tonsillar $CD10^+$ GC B cells were transfected with a c-Myc expression plasmid (treatment) or empty vector (control). mRNAs from 8 independent treatment-control pairs (8 human tonsils) were subjected to gene expression profiling (Affymetrix® U133 plus2.0). Gene set enrichment analysis (GSEA) and bioinformatics integration of two large clinical lymphoma micorarray data sets were used to generate a c-Myc gene expression signature.
Results: Microarray profiled genes were ranked by concordance of their expression levels with those of c-Myc in both tonsillar B cells and tumours. Gene set enrichment analysis revealed a strong enrichment of c-Myc target genes and a depletion of CD40/NF-κB pathway targets. We defined the c-Myc signature comprising the top c-Myc responding genes as c-Myc index. This index stratifies aNHL patients based on the expression of the c-Myc signature genes. The signature is consistently expressed in BL, while its expression varies in DLBCL. In two independent clinical DLBCL microarray data sets the presence of a high c-Myc index is significantly associated with a shorter overall survival.
Conclusion: Our approach integrates two important aspects of cancer research; intervention in experimental model systems and observation on tumour samples. Mimicking aberrant c-Myc expression in GC B cells provided us with insights into downstream molecular pathways affected, confirmed BL as unique disease entity, and yielded a novel prognostic stratification of DLBCL.

418 Differences in radiation, cisplatin, and cetuximab sensitivity between subpopulations of head and neck cancer cells

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Background: Mortality in head and neck cancer is high due to the emergence of therapy resistant local and regional recurrences that may originate from resistant cancer stem cells (CSCs). Cells with CSC characteristics in head and neck squamous cell carcinomas (HNSCCs) have been identified, for example, by the expression of CD44. However, no definite marker of CSCs in HNSCC has been presented. The aim of the present study was to identify cells with CSC characteristics in HNSCC cell lines and evaluate differences in sensitivity to radiation, cisplatin and the EGFR antibody cetuximab between subpopulations of cancer cells.
Material and Methods: A HNSCC cell line established at the division of otorhinolaryngology at Linköping University Hospital was used in passages 8−20. Experiments were approved by Linköping ethical committee. Cells were cultured in Keratinocyte Media supplemented with 1% fetal calf serum. Subpopulations of cells were detected and sorted after direct immunofluorescence staining of surface CD44 (CD44 bright/dim cells) or epithelial growth factor receptor (EGFR+/− cells), or vital staining of aldehyde dehydrogenase (ALDH) activity (ALDH+/− cells). Sorted cells were exposed to radiation (4 Gy), cisplatin (2 μg/ml for 1 h) or cetuximab (30 nM for 5 days). Ten days after sorting, treatment response was analyzed by a crystal violet assay.
Results: CD44 bright, EGFR−, and ALDH+ cells had a lower sensitivity to radiation, cisplatin and cetuximab compared to CD44 dim, EGFR+, and ALDH− cells. Differences in the morphology were found between CD44 bright and dim cells and between EGFR− and + cells, with CD44 bright and EGFR− cells displaying a more spindle shaped morphology. In contrast, ALDH+ cells were found to be larger and more round-shaped compared to ALDH− cells. Co-staining of the markers showed that the majority of EGFR− cells had a high CD44 staining while ALDH positive cells were found to be CD44 negative.
Conclusion: We here identify, in a recently established HNSCC cell line, subpopulations of cells using markers suggested to characterize CSCs; however, co-staining could not identify a single population of cells positive for all of these CSC markers. This may suggest heterogeneity among the CSC population. More importantly, all CSC-like subpopulations displayed a lower sensitivity to radiation, cisplatin and cetuximab compared to their respective control population.

419 V600EB-RAF cooperates in Epithelial to Mesenquimal Transition regulating E-Cadherin and ILK-1 expression through the MEK/ERK-MAPK pathway

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B-RAF mutations have a high prevalence in papillary thyroid cancer (PTCs) and anaplasic thyroid cancer (ATC), ranging from 35 to 65%. The most commonly B-RAF genetic alteration in human PTCs is a thymidine to an adenosine transversion at nucleotide 1799 that leads to a V600E substitution. This ^{V600E}B-RAF mutation is important not only to initiate tumourogenesis, but also required for the maintenance and progression of PTCs to advanced stages of tumour, extrathyroidal invasion and metastasis, as well as correlated with increased levels of Epithelial to Mesenchymal Transition(EMT)-associated genes.
On the other hand, Integrin Linked Kinase (ILK) has been also linked to cancer, because its expression is also elevated in tumours and correlates with tumour stage and grade. Thus, although ILK is expressed in normal thyroid cells, its levels are much higher in thyroid tumours. Its role could be exerted through the down-regulation of E-Cadherin expression, leading to the subsequent EMT.
The aim of this work is to study new molecular mechanisms mediated by ^{V600E}B-RAF in the development of PTC and elucidate the implication of this mutation in the regulation of certain proteins involved in the EMT. For this purpose we used three thyroid cancer cell lines, two of them harbouring the ^{V600E}B-RAF mutation and another one expressing wild type B-RAF.
We observed that ^{V600E}B-RAF abrogation by using short interfering RNA, reduces the expression of ILK, measured by quantitative real time PCR assays and western blot. This decrease correlates with lower phosphorylation levels of