cytokines and chemotherapeutic agents on growth and gal-1 expression was explored in tumour cells and xenografts by Western-blotting, IHC/ICC, real-time PCR and FACS.

Results: Gal-1 overexpression was detected in HL cells and also in the extracellular matrix of HL, especially in the nodular sclerosis subtype. Gal-1 expression and regulatory T-cell phenotype were observed in corresponding regions in different HL subtypes. Lymphoma/leukaemia cell lines expressed different levels of gal-1 (from zero to high). Gal-1 was overexpressed in the KMH2 cell line and xenograft. Treatments (mycophenolate-mofetil, rapalogs, TGFb, PI3K-inhibitor, cyclophosphamide) have been initiated in *in vitro* and *in vivo* HL models in order to study their effect on proliferation and gal-1 expression.

Conclusion: Our data suggests that gal-1 overexpression may be of importance in Hodgkin-lymphoma, presumably by promoting the survival of tumour cells. Certain subtypes of Hodgkin-lymphomas contain relatively few tumour cells compared to the microenvironment, which suggests a remarkable interaction between these components, perhaps modulated by gal-1. Therefore, a more thorough knowledge of the regulation of gal-1 expression and function is required using HL-samples, *in vitro* and *in vivo* HL-models.

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560 BRCA1 protein expression correlates with cancer stem cell markers in primary breast cancer

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Background: Based on Cancer Stem cell (CSC) hypothesis cancers originate in tissue stem or progenitor cells through the dysregulation of self-renewal process. Breast cancer cells with a $CD44^+/CD24^{-/low}$ phenotype have been proposed to have tumour initiating properties with stem cell-like and invasive features. BRCA1is an important susceptibility gene for breast cancer which plays a crucial role in DNA repair, activation of cell-cycle checkpoints, and maintenance of chromosome stability. The clinical, molecular, and pathologic features of breast cancer in BRCA1 mutation carriers suggest that BRCA1 may function as a stem-cell regulator.

Material and Methods: The purpose of the current study was to investigate the relationship between BRCA1 protein expression and clinicopathological characteristics, and putative cancer stem cell markers in a well-characterized series of unselected breast carcinomas. Immunohistochemistry was performed on 156 primary operable breast tumours using a monoclonal anti-BRCA1 primary antibody.

Results: Adjacent normal breast tissue showed strong BRCA1 immunoreactivity mainly localized to nuclei of the cells with no cytoplasmic staining. In breast cancers, complete loss of nuclear expression was observed in 23 cases (15%), whereas cytoplasmic expression was found in 133 breast carcinomas (85%) which was correlated with nuclear pattern. Absent or reduced nuclear BRCA1 expression was observed more frequently in invasive ductal carcinoma, and less frequently in medulary carcinoma and lobular carcinomas. (p-value = 0.005).

Altered BRCA1 expression was significantly associated with high grade and poor prognosis breast tumours (p value = 0.006). It was also more often seen in early onset breast cancer patients ($\leqslant$40 years) rather than patients over age of 40 (p value = 0.04). We further established a significant correlation between the BRCA1 expression levels and the CD44+/CD24− cancer stem cell phenotype in primary breast tumours (P = 0.02).

Conclusion: Taken together, our results indicate that loss of BRCA1 expression is a marker of tumour aggressiveness, potentially linked to BRCA1 status and a cancer stem cell phenotype in primary breast cancer. Breast cancer stem cells are more likely than non stem cell to have low levels of BRCA1 expression. These finding support the idea that loss of BRCA1 expression may result in an accumulation of genetically unstable breast stem cells, providing targets for further carcinogenic events.

561 Renin-angiotensin system expression in myeloproliferative diseases

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Myeloproliferative diseases present the group of clonal malignant diseases of hematopoietic stem cell. Somatic mutation of *JAK2* gene ($JAK2V617F^+$) is present in most of the patients (>90%) with polycythemia vera (PV), and 50% of patients with essential thrombocythemia (ET). This mutation causes the constitutive activation of tyrosine kinase and the consequence is cytokine independent proliferation of cells. Signaling pathway JAK2/STAT5/Bcl-xL is essential for erythropoiesis, controlling cell proliferation and survival. In $JAK2V617F^+$ PV and ET, growth of erythroid progenitors is erythropoietin independent. There is a lot of evidence of local renin-angiotensin system (RAS) presence in bone marrow affecting cell proliferation and differentiation. There is an increase of mRNA expression of angiotensinogen (AGT), rennin (REN) and angiotensin II receptor 1 (AT2R1) in bone marrow of $JAK2V617F^+$ PV and ET patients. Our research is focused on understanding the correlation of these two pathways in order to find the control points that can be used as a drug targets for myeloproliferative disorders.

Bone marrow mononuclear cells from PV and ET patients were seeded on MethoCult (StemCell) in medium with and without erythropoietin (EPO). At day 13 erythroid colonies were observed for morphology differences, colony density and isolation of DNA, RNA and proteins (Trizol, Invitrogene). DNA is used to determine JAK2 status by allele-specific PCR, RNA for real-time PCR detection of RAS components and proteins for Western detection of AT2R1 protein.

We analyzed 5 different bone marrow samples by now – 3 PV ($JAK2V617F^+$), 1 PV ($JAK2V617F^-$) and 1 ET ($JAK2V617F^+$). $JAK2V617F^+$ PV and ET erythroid colonies grown without EPO were smaller (50–100 cells), paler and in lower density then erythroid colonies grown with EPO (>200 cells). No erythroid colonies were noticed in $JAK2V617F^-$ PV sample without EPO. We collected high quality DNA, RNA and protein from cca 10^4 cells. We modified protein extraction method to gain better solubilization of proteins by resuspending them in 2% DEA. Preliminary results suggest increase of AT2R1 expression in $JAK2V617F^+$ patients when compared with $JAK2V617F^-$ patients. Western and RT-PCR data are in preparation.

Confirmation of higher expression of RAS components in patients that have constitutive activation of JAK2 will enable us to use drugs like ACE inhibitors and AT2R1 antagonists in assessing erythroid proliferation-differentiation process.

562 Breakpoints at 17p11.2 detected by high-resolution 500K SNP arrays identifies most metastatic colorectal carcinomas

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Background: The genetics of metastatic colorectal cancer have been studied for many years using a variety of techniques with limited resolution which hampers identification of specific underlying cancer-associated genes. Introduction of high-density single nucleotide polymorphism arrays has allowed identification of small regions of chromosomal gains and losses with a much higher resolution of down to 2.5 kb.

Material and Methods: Here we used 500K SNP mapping arrays to map the overall genetic lesions present at diagnosis in 23 primary sporadic CRC patients with liver metastasis. In order to evaluate the consistency of the chromosomal changes identified by the SNP-arrays, interphase FISH analysis were performed in parallel for a total of 24 chromosome regions from 20 different chromosomes.

Results: The highest frequency of copy number (CN) losses detected corresponded to chromosomes 1p (n = 17; 74%), 8p (n = 18; 78%), 14q (n = 15; 65%), 17p (n = 19; 83%), 18 (n = 21; 91%) and 22q (n = 17; 74%) while CN gains more frequently involved chromosomes 1q (n = 10; 43%), 7 (n = 20; 87%), 8q (n = 17; 74%), 13q (n = 18; 78%), 20q (n = 20; 87%) and X (n = 13; 57%). SNP arrays allowed the identification of small (<1.3 Mb) and extensive/large (>1.5 Mb) altered DNA sequences. Interestingly, several of these regions contain cancer genes known to be involved in colorectal cancer and metastatic process (particularly among the amplified chromosome regions).

Conclusions: Overall our results showed a high degree of correlation between both methods, including for the most frequently altered regions. Moreover, four recurrent chromosomal breakpoints were identified at chromosome 1p12, 8p12, 17p11.2 and 20p12.1. Interestingly, detailed analysis of recurrent chromosomal breakpoints reveals a highly prevalent breakpoint at 17p11.2 which may target genes such as the *FAM27* gene whose role in the disease deserves further investigation.