685 BCL11B overexpression leads to DNA damage resistance and delayed cell cycle progression

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Background: The expression of *BCL11B* was identified in cells derived from T-cell lineage, neurons, keratinocytes and recently in a subset of squamous cell carcinomas. The depletion of *BCL11B* resulted in significant reduction of cellular survival in immature thymocytes, transformed T-cell and keratinocyte cell lines accompanied by replication stress and increased sensitivity to DNA damage. The ectopic overexpression in HeLa cells and a hematopietic progenitor cell line caused cell cycle retardation of unknown origin. These features predispose *BCL11B* to function as an oncogene or a tumour suppressor. Genetic losses and inactivating mutations found in T-cell lymphomas in mice supported the tumour suppressive role. The amplifications and expression of wild-type *BCL11B* shown in human T-cell leukemias/lymphomas indicated its oncogenic potential. In order to better understand the role of *BCL11B* in the survival of human T-cell derived tumours we analyzed the effects of *BCL11B* accumulation on cell survival and cell cycle progression.
Materials and Methods: The retroviral-vector based *BCL11B* overexpression system was developed in Jurkat and huT-78 T-cell lines. The *BCL11B* deletion mutants were created using PCR. The influence of elevated Bcl11b on survival was assayed by treatment with radiomimetic drugs and TRAIL followed by Annexin-V binding assay. The DNA damage was measured by γ-H2AX detection. Cell cycle was monitored using propidium iodide staining and BrdU-incorporation assay.
Results: Forced overexpression of *BCL11B* resulted in increased resistance to radiomimetic drugs. No influence on death-receptor apoptotic pathway was observed. The apoptosis resistance was accompanied by the cell cycle delay caused by accumulation of cells at G1. The cell cycle restriction was associated with upregulation of CDKN1C (p57) and CDKN2C (p18) cyclin dependent kinase inhibitors. Moreover, p27 and p130 proteins were accumulated and the SKP2 gene responsible for their degradation was repressed. The expression of MYCN oncogene was silenced and resulted in significant depletion of the protein. The cell cycle delay and DNA-damage induced apoptosis resistance coincided and were dependent on the presence of the histone deacetylase binding N-terminal domain.
Conclusions: The data presented here reveal the potential role of *BCL11B* in promoting tumour survival and chemoresitance and encourage to develop *BCL11B*-inhibitory strategies as potential therapeutic approaches.

686 Recruitment of histone methyltransferases to the p53-MDM2 complex regulates p53 transcriptional output by promoting histone and p53 methylation

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p53 is a tumour suppressor protein which becomes directly deactivated in half of all human malignancies. The remaining half contains in turn other alternative lesions in the p53 pathway, such as the inordinate activation of the p53 negative regulator MDM2.
p53 acts as a transcriptional activator of numerous genes implicated in stress responses, such as DNA repair, cell cycle arrest, and programmed cell death. Consistently with its role, p53 is known to interact not only with the transcriptional machinery components but also with chromatin modifiers. Analogically, MDM2 can function as a transcriptional repressor capable of recruiting histone deacetylases and corepressor proteins to chromatin and p53.
Here, we report that p53 and MDM2 interact with repressor histone methyltransferases SUV39H1, EHMT1/GLP and EHMT2/G9a.
MDM2 mediates formation of p53-SUV39H1/EHMT1 complex capable of methylating histone 3 *in vitro* and binding to p53 responsive elements *in vivo*.
MDM2 additionally recruits EHMT1 to methylate p53 on K373.
Furthermore, knockdown of methyltransferases increases p53 activity in stress response and significantly reduces cell proliferation in Nutlin-3a dose-dependent manner.
Most importantly, knocking down of SUV39H1 accelerated induction of cell cycle arrest and appearance of SA-beta-Gal positive staining in a cellular model of p53-induced senescence.
We postulate therefore the MDM2-mediated recruitment of histone methyl-transferases to p53 as a novel mechanism of p53 regulation, important for cell cycle arrest response and cellular senescence.

687 The role of WWOX, a tumour suppressor gene in breast cancer – a microarray study of MDA-MB-231 cell line

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Background: *WWOX* is a tumour suppressor gene, located at 16q23.3–24.1, which spans the region of FRA16D – one of the common fragile sites. Changes in the *WWOX* coding region are the most common genetic changes in breast cancer – deletion in this area is observed in more than 80% cases of this type of tumour.
Suppressive character of *WWOX* gene has been confirmed in numerous studies. It has been demonstrated that increased expression of *WWOX* in breast cancer cell line MDA-MB-231 inhibits cell proliferation in suspension and reduces tumour growth rates in xenographic transplants. At the same time higher level of WWOX enhances cell migration through the basal membrane and changes morphology of colonies formed in Matrigel.
Reduced expression of *WWOX* in breast cancer patients, correlates with more aggressive course, higher relapse rate and higher mortality.
Materials and Methods: Initial experiments were performed on Human Discover Chips™ (ArrayIt®), containing 380 genes involved in major cellular pathways. The experiment confirmed altered expression of cell structure, proliferation and differentiation genes. The study was than extended to whole genome microarray analysis, in which Human OneArray™ (Phalanx Biotech), containing 30 985 probes, were used. For both experiments human breast cancer MDA-MB-231 cells were transduced with WWOX cDNA. Verification of obtained results was done by real-time RT-PCR. Additional validation will be performed by means of quantitative methods, enabling protein level measurements.
Results: Analysis of the microarray results, not only confirmed literature reports, concerning *WWOX* participation in Wnt/β-catenin pathway inhibition, but also allowed the identification of other differently expressed genes involved in key biological pathways. Differential expression of over 900 genes was found significant ($p < 0.05$). According to the molecular function, numerous transcription factors, signaling molecules, kinases, and numerous cytoskeletal proteins were identified.
Conclusions: On the basis of obtained microarray results, we concluded that *WWOX* takes part in differentiation and breast tissue remodeling. Due to differential expression of numerous cytoskeletal proteins and based on the data obtained from biological experiments, we presume that *WWOX* may be involved in formation of normal mammary gland structures. Restoration of WWOX cellular functions suppress cancer specific phenotype and leads to lowered tumourigenicity of MDA-MB-231 cell line.

688 Epigenetic dysregulation of hsa-miR-9 in hepatocellular carcinoma

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Introduction: Errant epigenetic modifications and subsequent dysregulation of microRNAs (miRs) have been frequently reported in hepatocellular carcinoma (HCC). This study aimed to study epigenetic dysregulation of hsa-miR-9 in HCC.
Material and Method: A panel of hepatoma cell lines and a non-tumourigenic liver cell line MIHA was treated with 5-aza-2′-deoxycytidine (5-aza-dC) and/or trichostatin A (TSA). Difference in mature miR-9 levels between the treatments, and between 65 paired human HCC samples, were assessed by quantitative reverse transcription-PCR (qRT-PCR). The expression of specific pre-miR-9 isoforms *in vitro* was assessed by semi-qRT-PCR.
Different 5′ regions of miR-9 in cell lines and clinical samples were amplified by bisulfite specific PCR (BSP), and the degree of amplicon methylation was assessed by taqI digestion. The true percentage of methylation was quantitated by sequencing.
Results: Upon 5-aza-dC addition, miR-9 upregulation, compared with the mock controls was observed in MIHA & 7 hepatoma cell lines. Also, hepatoma cell lines generally had higher levels of miR-9 than MIHA. RT-PCR of pre-miRs in MIHA & PLC suggested that the extra miR-9 was mainly constituted by miR-9−3. Assessment of clinical HCCs also showed that the majority that presented an actual difference between paired samples of the same case were neoplastic overexpressors, where tumour samples showed higher miR-9 levels.
DNA methylation of the previously documented promoter region of miR-9−3 did not correlate with the expression profile of MIHA, PLC & HLE. Instead the promoter may locate within another CpG island further upstream. Bisulfite sequencing of this region confirmed an agreement between 5-aza-dC expression profile and DNA methylation status.
MIHA, Bel7402 and SMMC, in the presence of TSA alone, can substantially increase miR-9 expression, suggesting that while DNA methylation played a great role *in vitro*, the effects of histone acetylation were also important

in miR-9 regulation. Histone reacetylation may explain what was observed clinically, where negligible difference of DNA methylation was noted between tumour & non-tumour sections in the neoplastic miR-9 overexpressors.
Conclusion: We present evidence of hsa-miR-9 upregulation, miR-9−3 in particular, via epigenetic means in HCC. We also propose a previously unreported promoter region for primary miR-9−3.

689 Investigation into the differential expression of early growth response-1 in diseased colon

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Early growth response (EGR1) is a zinc-finger transcription factor involved in the regulation of cell growth (Sukhatme, Cao et al. 1988; Pavletich and Pabo 1991). It can act as a tumour suppressor or a tumour promoter with a role in the induction of apoptosis in cancer cells by various pathways (Ham, Eilers et al. 2000; Thiel and Cibelli 2002). EGR1 appears to play a significant role in colorectal carcinogenesis and inflammatory pathways, suggesting a role in Inflammatory Bowel Disease (IBD) (de Mestre, Rao et al. 2005; Mostecki, Showalter et al. 2005; Annese, Valvano et al. 2006). Patients with IBD have a greated risk of developing CRC, which is increased with symptom duration, severity of inflammation and dysplasia (Munkholm 2003). The aim of this study was to determine if *EGR1* is differentially expressed in diseased colon tissue.
The relative *EGR1* expression was determined by qRT-PCR in a number of colorectal tissue samples: colorectal cancer (CRC) cell lines, matched normal mucosa and tumours from cancer patients, and matched IBD patient samples, some of which had been stimulated with inflammatory mediators (LPS, TNF, MDP and PGN), and mucosa from healthy controls. Statistical analysis of the data was performed using 'R' (R Development Core Team, 2009), using the Student's t test and the Kruskal-Wallis test. Statistical significance was set at <0.05. To determine if the methylation status of the *EGR1* promoter influenced expression, the promoter region was investigated using bisulfite sequencing.
The CRC cell lines were analysed to determine their relative *EGR1* mRNA expression levels, and showed very little *EGR1* expression, indicating that *EGR1* is down-regulated in CRC. Differential expression of *EGR1* was evident between 27/30 matched normal and tumour samples, with 12 patients showing a significant decrease in *EGR1* in the tumour and 15 patients showing a significant up-regulation in *EGR1*. *EGR1* is significantly down-regulated in IBD patients compared with healthy controls. Induction of *EGR1* by inflammatory stimuli also appears to be aberrant in the Crohn's disease patients. The differential expression of *EGR1* was not caused by aberrant methylation of the *EGR1* promoter in either the CRC or IBD patients.
This data provides clear evidence that *EGR1* is differentially regulated in both CRC and IBD, and in the case of Crohn's disease shows aberrant inflammatory response, suggesting that *EGR1* may play a role in both of these colorectal diseases.

690 MUC1 protein over-expression is mediated by MUC1 gene amplification in invasive breast carcinoma cells

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The Mucin 1 gene (MUC1), which is located on chromosome region 1q21.3-q22, is aberrantly over-expressed in approximately 90% of human breast cancers. Several studies have shown that MUC1 over-expression is due to transcriptional regulatory events. However, the importance of gene amplification as a mechanism leading to the increase of MUC1 expression in breast cancer has been poorly characterized. The aim of this study was to evaluate the role of MUC1 gene amplification and protein expression in human breast cancer development. Using real-time quantitative PCR (Q-PCR) and immunohistochemistry (IHC) methods, 89 breast tissue samples were analyzed for MUC1 gene amplification and protein expression. Q-PCR analysis showed MUC1 genomic amplification and a positive association with the histopathological group in 12% (1 out of 8) of benign lesions and 38% (23 out of 60) of primary invasive breast carcinoma samples ($p = 0.004$). Array-CGH meta-analysis of 886 primary invasive breast carcinomas obtained from 22 studies showed MUC1 genomic gain in 43.7% (387 out of 886) of the samples. Moreover, we identified highly statistical significant association between MUC1 gene amplification and MUC1 protein expression assessed by IHC and western-blot ($p < 0.0001$). In conclusion, this study demonstrated that MUC1 copy number increases from normal breast tissue to primary invasive breast carcinomas in correlation with MUC1 protein expression.

691 Function of the Pit-1 transcription factor in breast cancer

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The transcription factor Pit-1 plays a critical role in cell differentiation during organogenesis of anterior pituitary. However, Pit-1 is present in others tissues such as the mammary gland. In this gland, Pit-1 expression is higher in breast carcinomas with respect to normal breast. To study the role of Pit-1 in the mammary gland, we overexpress or knock-down Pit-1 in human mammary cell lines and mice, and evaluate cell proliferation, apoptosis, colony formation, cellular invasiveness, and tumoural development.
The non-invasive (MCF-7) and the invasive (MDA-MB-231) human breast adenocarcinoma cell lines were transfected either with the Pit-1 overexpression vector (pRSV-hPit-1) or Pit-1 siRNA. Western blots to evaluate Pit-1, cyclin D1, Bcl-2, PARP, E-cadherin, and Snail expression were carried out. Cell proliferation and apoptosis were assessed by BrdU incorporation and flow cytometry, respectively. Colony formation was carried out using soft agar. The cellular invasiveness was performed by using matrigel invasion chambers. We also evaluated tumoural growth and presence of metastasis in lung in SCID mice after injection of MCF-7 cells stably transfected with the Pit-1 overexpression vector.
Pit-1 overexpression in the MCF-7 cells increases proliferation, reduces apoptosis (by inducing cyclin D1 and Bcl-2 expression, respectively), increases cellular invasiveness, and colony formation, and inhibits E-cadherin by raising Snail expression protein levels. Endogenous Pit-1 knockdown in MDA-MB-231 cells reduces proliferation, colony formation, cellular invasiveness, and Snail protein expression, increasing E-cadherin expression. *In vivo*, Pit-1 overexpressing MCF-7 cells injected in immunodeficient mice increase tumoural growth, and induce morphological and biochemical changes towards mesenchymal characteristics. In addition, SCID mice orthotopically injected with the Pit-1 overexpressing MCF-7 cells into the mammary gland develops micro metastasis in lung.
All together, our data suggest that deregulation of the Pit-1 transcription factor in breast could be involved in mammary carcinogenesis and development of metastasis in lung.

692 Expression and potential role of SOX2 gene in human thymus and thymomas

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Background: SOX genes are expressed in a restricted spatial and temporal manner and are strongly involved in stem cell biology, organogenesis and human development. Indirect evidences in experimental animal models suggested a role for SOX2 in the thymus development. The aim of the present study is to investigate the expression and distribution of SOX2 immunoreactive cells in human normal thymus and thymomas.
Material and Methods: Five normal human thymuses and 10 thymomas were stained for SOX2 protein by using sensitive ADVANCE/HRP® biotin free immunohistochemical detection method. Presence, distribution and association of Sox2 expression with histopathology were observed in specimens included in the study. The local research ethic committee approved the protocol of the study, and informed consent was obtained from all subjects.
Results: Two expression patterns of SOX2 were found in human fetal thymus and thymoma. Nuclear expression was detectable in both normal thymus and thymomas, whereas cytoplasmic distribution alone or associated with nuclear pattern was demonstrated in thymomas only. In human fetal and postnatal thymus SOX2-positive epithelial cells were distributed in the thymic cortex, medulla and cortico-medullary junction of human fetal thymus, where cells with nuclear positive staining were grouped in small clusters as well defined networks. In postnatal normal human thymus, epithelial cells of the Hassall corpuscles strongly expressed SOX2. A heterogeneous pattern of distribution of SOX2 was recognizable in thymoma. In B3 thymoma, immunoreactivity was observed in malignant epithelial cells, and also in endothelial cells of intratumoural blood vessels.
Conclusions: In this study for the first time we have described the presence of SOX2-positive cells in the normal and pathologic human thymus, and we have suggested an its potential oncogenic role in the development of thymoma. Our evidence of the presence of SOX2-positive cells inside the tumour vascular endothelium in type B3 thymoma specimens, suggests the hypothesis of a presence of an epithelial stem cell population able to differentiate in both endothelial and thymic malignant epithelial cells.