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