Conclusion: This study has identified novel prognostic markers for rectal cancer. Validation by bisulphite pyrosequencing in an independent cohort is underway. Analysis of global methylation changes in rectal cancer, not previously reported, has provided an insight into key pathway that is responsible in disease progression and may be target for future therapeutic studies.

1036 POSTER

Thymoquinone-induced UHRF1 Ubiquitination is a Key Event for Challenging Apoptosis in Cancer Cells

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Background: UHRF1 (Ubiquitin-like containing PHD Ring Finger), an anti-apoptotic protein essential for cell proliferation, is over-expressed in several types of cancer. UHRF1 participates in a huge macro-molecular complex including DNMT1 (DNA methyltransferase 1), Tip60 (a histone acetyltransferase), HAUSP (a ubiquitin specific protease) and HDAC1 (a histone deacetylase). It has been shown that HAUSP protects, *in vitro*, UHRF1 from auto-ubiquitination and regulates its stability *in vivo*. We previously showed that thymoquinone (TQ), an anti-cancer drug, induced UHRF1 down-regulation by a p73- and caspase 3- dependent process. The goal of the present study was to determine more precisely the pathway involved in the degradation of UHRF1 by TQ.
Material and Methods: Jurkat cells (T lymphoblastic leukaemia cells) and human astrocytoma cells (cell line U87) were used as cancer cell models. Western blot experiments were performed to detect UHRF1, HAUSP and p73 in both cell lines.
Results: We have observed that TQ induced a dose-dependent down-regulation of UHRF1 and HAUSP accompanied with p73 up-regulation. Interestingly, kinetic study revealed the presence of higher bands of UHRF1 expression as assessed by western blotting on both cell lines. Co-immunoprecipitation experiments allowed us to demonstrate, in Jurkat cells, that these bands were due to ubiquitination of UHRF1. These data show that the degradation of UHRF1, challenged by TQ, is due to its ubiquitination through an as yet unknown mechanism but which appears dependent upon HAUSP down-regulation.
Conclusion: In conclusion, we propose that UHRF1 ubiquitination is a key event in the TQ-induced apoptosis in cancer cells. This ubiquitination might result from the auto-ubiquitination activity of UHRF1, following HAUSP down-regulation.

1037 POSTER

The Prolyl-3-hydroxylases (P3H) and P3H-related Genes CRTAP and SC65 Are Novel Transcriptionally Silenced Genes in Burkitt's Lymphoma

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Background: A number of genes are subject to epigenetic inactivation in Burkitt's lymphoma (BL). Hydroxylation at proline residues is a critical post-translational modification in the biosynthesis of collagen and this mediated by a number of prolyl hydroxylases which act at the 3 and 4 proline positions. Here, we show that the collagen prolyl 3 hydroxylases (P3H) *Leprecan* (P3H1, Lepre), *Leprel1* (Leprecan like1, P3H2) and *Leprel2* (P3H3) and the P3H paralogs, Cartilage Related Protein (*CRTAP; Leprel3*) and Synaptonemal Complex 65 (*SC65; Leprel4*) are targets for epigenetic inactivation in BL.
Material and Methods: We used RT-PCR, immunohistochemistry, methylation specific PCR (MSP) and pyrosequencing to analyse expression and methylation level of the P3H and P3H-like genes in BL cell lines and primary lymphoma biopsies.
Results: Methylation in each of the P3H and P3H-like genes is detected in BL cell lines and primary lymphomas and correlates well with down-regulation of expression. In contrast, the CpG islands are unmethylated or methylated at lower levels in DNA isolated from bone marrow of healthy individuals and in lymphoblastoid cell lines. Of note, there is simultaneous methylation of Leprel1, Leprel2 and SC65 in many BL cell lines and primary BL, implying that the three genes encode proteins with at least partially non-redundant functions. Methylation of Leprel1 and Leprel2 occurs in both sporadic BL and in BL associated with immuno-suppression.
Conclusions: The frequency of transcriptional silencing of the P3H and P3H-related genes in BL, taken together with their known biological properties, implies that prolyl hydroxylation has important functions in lymphoma suppression, loss of which is important in lymphomagenesis.

1038 POSTER

Promoter Hypermethylation of APC, P16, and RASSF1a Genes in Gastrointestinal Cancer

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Background: Gastrointestinal is the most common cancer type in the world. Cancer is a disease involving dynamic changes of genetics and epigenetics and the formation of tumour is a complex and multi-step process. Abnormalities of DNA promoter hypermethylation which is an epigenetic alteration plays an important role in carcinogenesis. DNA methylation profile is useful marker for tumour diagnosis.
Materials and Methods: 20 tumour tissue samples were collected by endoscopy and/or colonoscopy and 20 blood samples were collected from healty people. After bisulfite modifications of these samples, MSP (Methylation Specific PCR) analysis of *APC*, *p16,* and *RASSF1A* genes were performed.
Results: A relationship between promoter hypermethylation of *APC*, *p16,* and *RASSF1A* genes and tumour progression was found.
Conclusions: Our study suggests that promoter hypermethylation of tumour suppressor genes, such as *APC*, *p16* and *RASSF1A*, is a useful molecular marker for gastrointestinal tumours.

1039 POSTER

Epigenetic Alterations in Lung Cancer Susceptibility Regions

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Background: Genome-wide association (GWA) studies have identified lung cancer susceptibility loci, but the corresponding causal mechanisms have not yet been unravelled. Epigenetic mechanisms play an important role in mediating environmental influences on gene expression, and promoter methylation is involved in lung carcinogenesis. Methylation of CpG islands in susceptibility loci was thus investigated as a promising biomarker of lung cancer susceptibility.
Material and Methods: Quantitative high throughput methylation and genotype analyses using MALDI-TOF (Sequenom) were performed. A discovery (n = 34) and a validation (n = 48) set of non-small cell lung cancer (NSCLC) tumours and adjacent normal lung tissue were assessed for methylation in CpG islands located within three lung cancer susceptibility loci. Subsequently, blood samples from lung cancer cases (n = 890) and controls without lung cancer (n = 510), were analysed for *TERT* promoter methylation. A comparative genome-wide methylation analysis after MCIp-enrichment was performed on blood samples from lung cancer cases and controls using whole genome CpG island arrays (Agilent). RT-PCR and *in vitro* expression analyses were also performed.
Results: Tumour hypermethylation was found for the *CHRNA3* and *TERT* promoter CpG islands, while the *CHRNB4* promoter and *TERT* gene body CpG islands were hypomethylated in tumours vs. adjacent normal lung tissue ($p < 001$). Expression data broadly correlated with the observed methylation. Methylation at one *TERT* promoter CpG unit correlated with the genotype at rs421629 ($p = 0.002$). In blood samples of cases compared to controls, the *TERT* amplicon investigated showed a statistically significantly increased average methylation ($p < 0.0001$). In the genome-wide methylation screen, *TERT* was one of the genes identified as differentially methylated between cases and controls.
Conclusions: *CHRNA3*, *CHRNB4* and *TERT* are putative lung cancer susceptibility gene previously identified by GWA studies. This work shows that they are epigenetically dysregulated in lung tumours. The association of tumour-specific *TERT* methylation with *TERT* genotype points to a possible mechanism of the association with lung cancer risk for this locus. The increased methylation found in blood samples from lung cancer cases compared to controls supports *TERT* methylation as an epigenetic marker for early diagnosis. Further investigations are required to determine its value as a susceptibility marker.