716 CpG island hypermethylation of 35 tumour suppressor genes in endometrioid endometrial carcinomas

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Background: A subset of endometrial carcinomas has methylation of the CpG islands in the promoter region of several tumour suppressor genes. However, methylation patterns and pathophysiological consequences are not well characterized.
Material and Methods: Samples from 36 endometrioid endometrial carcinomas (EEC) were retrieved from the Tumour Bank and the Surgical Pathology files of Hospital de la Santa Creu i Sant Pau, Barcelona, Spain. Genomic DNA was extracted from frozen tumour. Methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) was used to assay CpG island methylation status of 35 different tumour suppressor genes (MRC-Holland®). This method is based on probes that recognize specific sequences in DNA containing a restriction site for a methylation sensitive HhaI enzyme. Methylation specific PCR (MSP) was performed to validate the methylation patterns. DNA was subjected to sodium bisulfite modification using the Methylamp One-Step DNA Modification kit (Epigentek®), and then was subjected to MSP using specific primers for detection of methylated and unmethylated DNA. Results were correlated to various genetic alterations including microsatellite instability (MI), *PTEN, PIK3CA, K-RAS*, CTNNB1, and p53, with the clinicopathologic parameters, and patients follow-up.
Results: Fifteen genes were found methylated in at least 10% of tumours. Promoter hypermethylation was found more frequently in *CDH13* (97%, 35/36), and *RASSF1A* (83%, 30/36) than *MGMT* (53%, 19/36), *MLH1* (50%, 18/36), *WT1* (47%, 17/36), *PAX5* (33%, 12/36), *TIMP3* (31%, 11/36), *TP73* (31%, 11/36), *APC* (28%, 10/36) or *MSH6* (25%, 9/13). Promoter hypermethylation of *TP53*, *GATA5*, *CHFR*, *CD44,* and *CASP8* was found in less than 20% of cases. A CpG Island Methylator Phenotype (CIMP) (methylation of at least 8 of 35 genes) occurred in 33% (12/36) of EEC. The *MLH1* methylation status divided the tumours in high (CIMP) versus low methylation groups ($p = 0.00$). Inactivation of *MHL1* by promoter hypermethylation was present in most carcinomas with MI ($p = 0.002$). Moreover, a trend of inverse correlation was found between *MLH1* promoter hypermethylation or CIMP tumours and *PIK3CA* mutations ($p = 0.060$, and $p = 0.115$ respectively).
Conclusions: *MLH1* methylation may be a general predictor of CIMP. Moreover, the inverse correlation between *MLH1* promoter hypermethylation or CIMP tumours and *PIK3CA* mutations might favor the occurrence of two subgroups of endometrioid endometrial carcinomas.

717 CDX2 regulation in intestinal metaplasia of the stomach

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Background: Intestinal metaplasia (IM) of the stomach is a preneoplastic lesion that appears following *Helicobacter pylori* infection and that confers increased risk for gastric cancer development. IM is induced by *de novo* expression of the intestinal-specific transcription factor CDX2 in the gastric mucosa. However, the regulatory mechanisms and molecular pathways involved in the triggering and maintenance of CDX2 expression in the stomach are yet to be fully unravelled.
Methods: A cell culture and transfection approach was used, together with patient's tissue samples histopathological and immunohistochemical analysis.
Results: Following the demonstration of the importance of the BMP pathway in normal intestinal differentiation, we were able to show that key elements of this pathway not only co-localized with CDX2 in IM but also positively regulated CDX2 in an in vitro context. Further, we showed that in Juvenile Polyps, an intestinal differentiation phenotype somehow inverse to gastric IM, loss of BMP pathway activity related with decreased CDX2 expression and loss of intestinal differentiation, thus reinforcing our previous results. Since CDX2 appears ectopically expressed in the stomach following *H. pylori* infection, we tackle the hypothesis of a direct regulation of CDX2 expression by bacterial interaction with epithelial cells in an *in vitro* co-culture model. Lastly, a "regulatory haplo-insufficiency" as well as an autoregulatory mechanism for CDX2 had been suggested. These results, taken together with the apparent stability of the metaplastic phenotype, led us to hypothesize that CDX2 is regulating its own expression through an autoregulatory loop. We were indeed able to demonstrate that not only CDX2 binds to and transactivates its own promoter but also it positively regulates its own expression in gastrointestinal human carcinoma cell lines.
Conclusion: Altogether, our results put forward some of the regulatory mechanisms involved in CDX2 regulation in the gastric context, thus contributing to unveil molecular pathways implicated in the establishment/maintenance of the Intestinal Mataplasia of the stomach.

718 Selective activation of p53 target genes by depletion of various RNA polymerase I transcriptional factors

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Background: The nucleolus is a key organelle that regulates the synthesis of ribosomal subunits. Recent report suggested that the function of the nucleolus is tightly linked to cell growth and apoptosis. A variety of cellular stresses induces nucleolar stress by disrupting nucleolar structure. We and other groups showed that abrogation of RNA polymerase I-dependent transcription in nucleoli induced p53 accumulation and its acetylation. The acetylated p53 then binds to the promoter region of p53 target genes and regulates their transcription.
The functional consequences of p53 acetylation suggested that the timing of acetylation of the different p53 regions may be important for accurate p53 regulation and cell fate determination. The activation of genes involved in cell cycle control requires partial acetylation, whereas the activation of proapoptotic genes requires full acetylation of p53.
Material and Methods: We screened for RNA polymerase I-transcription regulatory factors whose knockdown induce p53 activation in MCF-7 cells using siRNA library.
We generated siRNA against transcription initiation factor-IA (TIF-IA), UBF, TAF_I48, and CD3EAP.
The expression and acetylation levels of p53 were analyzed by immunoblot in TIF-1A, UBF, TAF_I48, or CD3EAP siRNA treated cells. Furthermore, we examined the induction levels of p53 target gene products by immunoblot and RT-qPCR.
Result and Conclusion: Because the RNA polymerase I-dependent transcription levels affects on p53 acetylation, we examined the relationship between the deficiency of RNA polymerase I-dependent transcription and p53 target gene selectivity. To investigate this, we knocked down several factors which are known to be involved in RNA polymerase I-dependent transcription: i.e. TIF-1A, UBF, TAF_I48, and CD3EAP. Interestingly, although depletion of these factors induced the defect of RNA polymerase I-dependent transcription, p53 accumulation, and its acetylation, there were differences in p53 target genes that were activated. The depletion of CD3EAP enhanced the expression of proapoptotic genes such as PUMA and NOXA. On the other hand, the depletion of TIF-IA increased the expression of p21, HDM2, and proapoptotic genes. Selective activation of p53 target genes caused by various nucleolar stresses may be due to the different way of inhibition of RNA polymerase I dependent transcription.

719 Conjugation of endogenous BRCA1 protein with SUMO-2/3 is cell cycle-dependent

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Background: BRCA1, the main breast and ovarian cancer susceptibility gene, has a key role in maintenance of genome stability, cell cycle and transcription regulation. Interestingly, some of the numerous proteins which interact with BRCA1 protein undergo conjugation with small ubiquitin-like modifiers (SUMO). This post-translational modification is related to transcription, DNA repair, nuclear transport, signal transduction, and to cell cycle stress response. These features of sumoylation mechanisms and of BRCA1 function lead us to test the hypothesis of BRCA1 sumoylation by SUMO-2/3 (mainly involved in cellular stress response).
Material and Methods: Protein sequence was analysed by the SUMOplot™ software. Nuclear extracts of MRC5 human embryo lung cell line, MCF7 breast cancer cells, DU145 prostate cancer cells were immunoprecipitated by anti BRCA1 antibodies and analysed by Western blot with anti SUMO2/3 antibodies. To reduce non specific interactions, the immune complexes had been washed in PBS plus 0.2M NaCl, and in PBS plus 0.1 M NaCl.
Results: Protein sequence analysis suggests that sumoylation target sites belong to the RING finger and BRCT domains (BRCA1 C-terminus), two crucial regions for BRCA1 function. Moreover SUMO interacting motifs are present in the sequence of almost all proteins of BRCA1 network. Immunoprecipitations and Western blotting show the conjugation of endogenous nuclear BRCA1 protein with SUMO-2/3. In DU145 cells, BRCA1 conjugation with SUMO-2/3 is linked to the cell cycle and seems to be related to the oxidative stress. No cell cycle dependence of sumoylation is observed in MCF7 cells.
Conclusions: Our preliminary data and a number of arguments are in favour of a conjugation between SUMO-2/3 and the RING finger and/or the C terminal BRCT repeat of BRCA1 protein. Depending on the cell line, this conjugation appears to be modulated by the cell cycle and seems to be related to the oxidative stress, although the mechanisms remain to be determined. BRCA1 sumoylation may have a general role in the building, stability and/or activity of macromolecular complexes, especially in the nucleus. BRCA1 conjugation with SUMO-2/3, its interaction with SUMO1 and SUMO modification of many