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

G. Gernon[1], E. Nimmo[2], M. Aldhous[2], A. Smith[2], M. Walker[1], J. Satsangi[2], M. Dunlop[1], S. Farrington[1]. [1]*Institute of Genetics and Molecular Medicine University of Edinburgh, Colorectal Cancer Genetics Group, Edinburgh, United Kingdom,* [2]*Institute of Genetics and Molecular Medicine University of Edinburgh, Gastrointestinal Unit, Edinburgh, United Kingdom*

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

M. Croce[1], E. Lacunza[1], M. Baudis[2], A. Colussi[1], A. Segal-Eiras[1], M. Abba[1]. [1]*CINIBA, Faculty of Medical Sciences UNLP, La Plata, Argentina,* [2]*Institute of Molecular Biology, University of Zurich, Zurich, Switzerland*

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

I. Ben-Batalla[1], S. Seoane[1], E. Arias[1], R. Gallego[2], T. García-Caballero[2], M. Macia[3], L. González[4], F. Vizoso[4], R. Pérez-Fernández[1]. [1]*School of Medicine, Physiology, Santiago de Compostela, Spain,* [2]*School of Medicine, Morphological Sciences, Santiago de Compostela, Spain,* [3]*School of Medicine, Obstetrics and Gynaecology, Santiago de Compostela, Spain,* [4]*Fundación Hospital de Jove, Unidad de Investigación, Gijón, Spain*

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

A.M. Cimpean[1], S. Encica[2], M. Raica[3]. [1]*Victor Babes University of Medicine and Pharmacy, Histology, Timisoara, Romania,* [2]*Niculae Stancioiu Heart Institute, Pathology, Cluj Napoca, Romania,* [3]*Victor Babes University of Medicine and Pharmacy, Histology, Timisoara, Romania*

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.