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ABSTRACT BOOK

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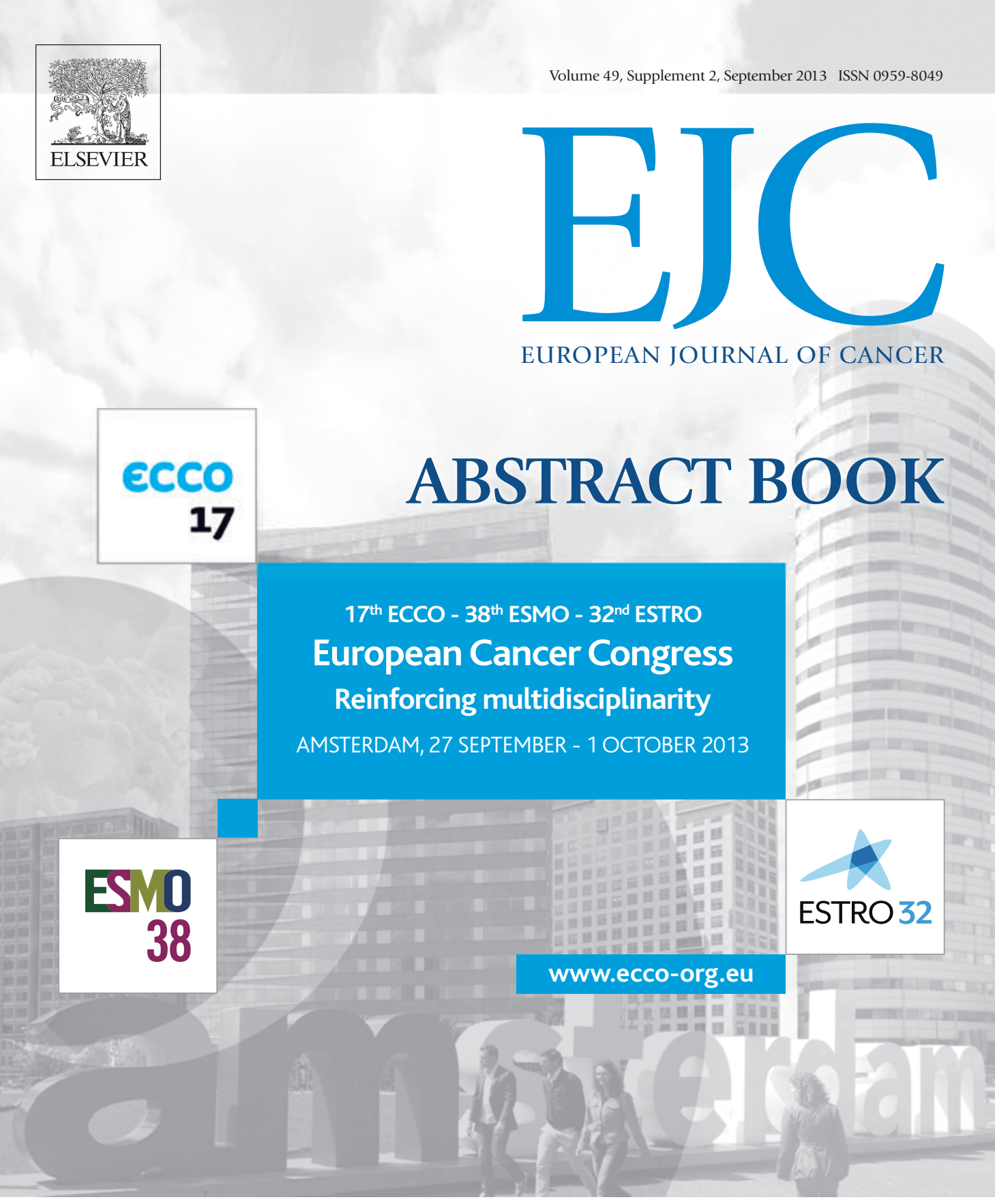

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679 POSTER

Potential functional role of EZH2 in endometrial carcinogenesis

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Introduction: Endometrial cancer represents the most common gynecological malignancy in the western world and the eighth leading cancer related cause of death in women. EZH2 is one potential novel target gene that might play crucial roles in endometrial carcinogenesis and metastasis. Functional *in vitro* analyses were designed to investigate potential alterations in expression profiles of EZH2. In detail, typical microenviromental epiphenomena of solid tumors (hypoxia, extracellular acidosis) as well as therapeutical approaches (hyperthermia) were mimicked in cell culture models. The polycomb group protein EZH2 acts as a transcriptional repressor and controls cellular memory and methylation processes. So far, the factor was found highly expressed in malignant vs. physiological tissues in several tumor entities. In particular, EZH2 over expression was described in various tumors with epithelial origin, e.g. endometrium, bladder, urothelial tissues, colon, esophagus, gastric tissue. Recently, it has been found that EZH2 overabundance is clearly associated with tumor aggressiveness and with an ascending histological grade and /or advanced stages of tumor progression, hence correlating with poor prognosis and reduced patient survival.
Materials and Methods: Endometrial cancer cell lines were cultured to more than 80% confluence. Experimental setup mimicked hypoxic conditions, extracellular acidosis or hyperthermia vs. regular conditions (control). mRNA expression levels of EZH2 were analyzed by quantitative RT-real-time PCR, followed by statistical analyses. Protein expression levels were determined by Western blot and immunocytochemistry.
Results: Acidic conditions as well as hyperthermia were identified as strong inductors of increased EZH2 expression levels in all cell lines tested. In contrast, hypoxia uniformly caused a down-regulation of EZH2 expression. EZH2 protein localization switched from complete nuclear expression under regular culture conditions to nearly complete deficiency of nuclear protein under hypoxia, acidosis and hyperthermia.
Conclusion: The obtained results clearly indicate the regulatory effects of acidosis, hypoxia and hyperthermal treatment on both, the mRNA and protein expression levels of EZH2. Thus, this factor might be of important relevance for tumor progression and metastasis with inferential therapeutic implications.
No conflict of interest.

680 POSTER

Variable DNA methylation profiles and protein expressions in breast cancer patients

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Background: During the process of tumorigenesis many tumour suppressor genes are inactivated that lead to the decreasing of relevant protein expressions and functions. The aim of this study was to investigate the role of epigenetic inactivation through evaluation of DNA methylation in promoters of 11 cancer associated genes in relevant protein expression changes in breast cancer patients. We selected genes responsible for self-sufficiency in growth signals (*ESR1*, *PGR B, RASSF1A, SOCS1, SYK and APC*) or inhibiting of cell invasion and metastases forming (*CDH1, TIMP3, ADAM23, CXCL12 and BRMS1*). Promoter methylation in these genes could cause decreasing of corresponding protein expression that will contribute to invazivity and metastasis forming processes in breast cancer.
Material and Methods: DNA methylation levels in paraffin embedded tumour tissues and blood cell samples from 34 patients with invasive breast carcinomas and peripheral lymphocytes from 50 control women were quantitatively evaluated by pyrosequencing. Protein expressions were estimated by immunohistochemical analyses using histoscore (intensity of staining x % of stained cells).
Results: The higher levels of promoter methylation in cancers were shown in *RASSF1A*, *APC*, *CXCL12*, *ADAM23 and PGR B* up to 86, 86, 64, 53 and 48%, respectively. The methylation levels in genomic DNA of patients and controls were significantly different in *APC*,*CXCL12*, *ESR1, PGR B and TIMP3* genes. Moreover, the significant differences between methylation levels in tumours and genomic DNA of patients were observed in *APC*, *ADAM23, CXCL12*, *ESR1, CDH1, RASSF1A, SYK, BRMS1* and *SOCS1* genes. Variable spectrum from high to none expressions were presented in tumour tissues in all of evaluated proteins; however, the significant negative association between protein expression and DNA methylation level in tumours was found for *APC* gene only.
Conclusion: DNA methylation profiles observed in our group of breast carcinomas are cancer specific, but they are not the only cause that affects the silencing of evaluated genes and decreasing of relevant protein products.
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No conflict of interest.

681 POSTER

Antidromic NFATc1 and p53 signaling at the edge of differentiation and stemness in pancreatic cancer

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Background: The current concept suggests a direct link between EMT and stemness induction in pancreatic cancer, thereby coupling cell motility and de-differentiation with self-renewal capacities and drug resistance. Both key features of cellular plasticity are controlled by distinct intracellular signaling and transcription pathways. We have shown that activation of the NFATc1 transcription factor promotes pancreatic cancer development and metastasis through its ability to integrate extrinsic stimuli into coordinated gene regulation.
Aim: To assess whether NFATc1 controls transcription of EMT genes and stemness in PDAC, particularly upon p53 inactivation.
Material and Methods: We generated mouse strains with combined pancreas-specific expression of NFATc1, $p53^{R172H}$ and $Kras^{G12D}$ using Cre-Lox technology. These mice showed a highly aggressive tumor growth (median survival of <50 days). Mouse primary tumour cells were used to identify NFATc1 targets by gene expression profiling and pathway analyses (ChIP seq, miRNA analyses and GSEA). NFATc1 mediated EMT and stemness were assessed in human and murine pancreatic cancer models using migration and spheroid assay as well as xenograft mouse models.
Results: Here, we identified antidromic NFATc1 and p53 signaling pathways in transcriptional control over EMT and stemness. We show that p53 activation prevents cells from EMT in a miR200 dependent manner. However, disruption of the tumor suppressor pathway enables NFATc1/Sox2 chromatin complex formation and transcription of EMT programmes, resulting in highly invasive and metastatic PDACs. Finally, re-expression of miR200c or NFATc1 inactivation suppresses EMT/stemness genes and re-sensitizes PDAC to chemotherapy.
Conclusion: Antidromic NFATc1 and p53 signaling pathways control key features of cellular plasticity and tumor progression at the level of gene transcription. These findings implicate key roles for NFATc1 in transcriptional regulation of differentiation and self-renewal in PDAC.
No conflict of interest.

682 POSTER

Genetic and pharmacological targeting of mast cells inhibits inflammation-associated gastric tumourigenesis

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Background: Mast cells (MC) are innate immune cells, which are important in microbial defence and allergic responses. The role of MCs in tumourigenesis and cancer progression is less well understood. Elevated MC numbers in the tumour stroma were correlated to human gastric cancer progression and promotion of angiogenesis. In this study we explore the role of MCs in tumourigenesis and tumour maintenance in a spontaneous mouse model for gastric cancer.
Material and Methods: Here we study the role of MCs in the $gp130^{FF}$ knockin mouse model, a validated preclinical model for Stat3-dependent inflammation-associated gastric cancer. MC-dependent tumour formation was investigated in the MC-deficient compound $gp130^{FF}$;$ckit^{wsh/wsh}$ mutant mice. To establish whether MCs are potential therapeutic targets, we also treated tumour-bearing $gp130^{FF}$ mice with either Cromolyn (MC degranulation inhibitor) or a dual inhibitor for the c-fms and c-kit receptor kinases (MC and macrophage (MΦ) inhibitor).