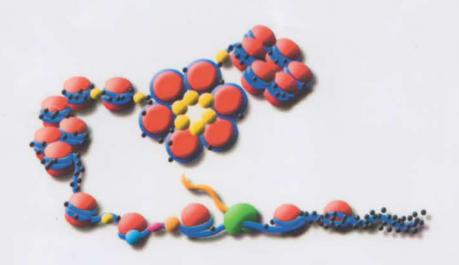
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results are being obtained with targeted therapies and immunotherapies. Staging of CMM patients relies essentially on clinico-pathological parameters, that, however, are not completely effective in identifying prognostic groups. This aggressive behavior of CMM is only partially explained by its genetic background, and accumulating evidence suggest an important role of epigenetic alterations in general, and of modifications of DNA methylation profiles in particular, in defining and modeling the biology of this malignancy. Indeed, CMM cells display an overall reduction in DNA methylation content as compared to normal melanocytes, and over 50 genes have been validated to be silenced by DNA methylation in CMM, affecting essentially every known cancer related pathway. This key role of altered genomic DNA methylation in CMM biology can offer important tools both for diagnosis/prognosis and for defining improved therapeutic strategies. Along this line, specific topics discussed will be: i) the role of methylation markers in defining prognosis of melanoma patients; ii) the impact of DNA methylation on immune recognition of CMM cells; iii) the effectiveness and safety of DNA hypomethylating drugs in modulating CMM immune profile; and iv) the use of DNA hypomethylating drugs in combined (immuno)-therapies. Comprehensively, the data available strongly support the exploit of CMM epigenetics to improve the management of patients affected by this malignancy.

ADAM23 methylation negatively associates with circulating tumour cells presence in breast cancer patients

Bozena Smolkova<sup>1</sup>, Michal Mego<sup>2</sup>, Iveta Zmetakova<sup>1</sup>, Viera Kajabova<sup>1</sup>, Tomas Krivulcik<sup>1</sup>,

Marian Karaba<sup>3</sup>, Juraj Benca<sup>3</sup>, Gabriel Minarik<sup>4</sup>, Tatiana Sedlackova<sup>4</sup>, Zuzana Cierna<sup>5</sup>, Ivana Fridrichova<sup>1</sup>

Laboratory of Cancer Genetics, Cancer Research Inst. of SAS, Bratislava, Slovak Republic; <sup>22nd</sup> Dept. of Oncology, Faculty of

Laboratory of Cancer Genetics, Cancer Research Inst. of SAS, Bratislava, Slovak Republic; <sup>2 2nd</sup> Dept. of Oncology, Faculty of Medicine, Comenius Univ., Natl. Cancer Inst., Bratislava, Slovak Republic; <sup>3</sup> Dept. of Surgical Oncology, Natl. Cancer Inst., Bratislava, Slovak Republic; <sup>4</sup> Institute of Molecular BioMedicine, Faculty of Medicine, Comenius Univ., Bratislava, Slovak Republic; <sup>5</sup>Inst. of Pathological Anatomy, Faculty of Medicine, Comenius Univ., Univ. Hospital, Bratislava, Slovak Republic

Circulating tumour cells (CTCs) in the peripheral blood or bone marrow are associated with metastatic potential in a variety of human cancers. Although DNA methylation of cancer-related genes is a promising diagnostic and prognostic biomarker, it was rarely studied in relation to the presence of CTCs. The aim of our study was to identify epigenetic changes in tumour samples that were associated with presence of CTCs in peripheral blood of breast cancer patients. DNA methylation in promoters of 11 genes responsible for self-sufficiency in growth signals (ESR1, PGR B, RASSF1A, SOCS1, SYK and APC) or regulating cell invasion and metastases formation (CDH1, TIMP3, ADAM23, CXCL12 and BRMS1) in 80 invasive breast carcinoma patients was analysed in our pilot study. Gene expression levels of KRT19, SLUG and TWIST genes in the CD34- enriched peripheral blood samples were analysed in order to identify presence of CTCs. CTCs were detected in 25% of patients (n=20). In their tumour samples we determined lower mean value of ADAM23 methylation compared to CTCs negative patients [5.1% (from 2 % to 21%) vs. 11.7% (from 2% to 60%); P=0.007]. However, we found no association between occurrence of CTCs and clinico-pathological characteristics of patients. ADAM23 gene is a member of ADAM (a disintegrin and metalloproteinase domain) family involved in various biological events such as cell adhesion, cell fusion, cell migration, membrane protein shedding and proteolysis. Recent studies have demonstrated that several ADAM genes are highly expressed in cancer cells and cancer tissues, although the exact roles of ADAM proteins, including ADAM23, in carcinogenesis remain largely unknown. On the other hand the aberrant DNA methylation was associated with ADAM23 expression silencing in breast, gastric and brain tumours. Therefore, the role of ADAM23 promoter methylation in invasivity and metastasizing processes need to be clarified by other studies.

## High Resolution Melting Analysis versus Pyrosequencing - Determination of the DNA Methylation Status of ESR2 in Tumor Tissue from Breast Cancer Patients

Melanie Spitzwieser <sup>a</sup>, Georg Pfeiler <sup>b</sup>, Margit Cichna-Markl <sup>a</sup>

Dept. of Analytical Chemistry, University of Vienna, Vienna, Austria; <sup>b</sup> Dept. of Obstetrics and Gynecology, Division of Gynecology and Gynecological Oncology, Medical University of Vienna, Vienna, Austria

Breast cancer is the most frequently diagnosed cancer type and the leading cause of cancer death in women worldwide. Aberrant DNA methylation patterns are considered as potential biomarkers for diagnosing breast cancer and/or for predicting the efficacy of breast cancer therapy. The present study focused on determining the DNA methylation status in the promoter region of estrogen receptor beta (ESR2) in tumor tissue from breast cancer patients. Two different methods were used: methylation sensitive high resolution melting (MS-HRM) analysis and pyrosequencing (PSQ). MS-HRM has a low limit of detection and is rather inexpensive. It can, however, not be applied to determine the methylation status of heterogeneously methylated templates. In addition, one only obtains the average methylation status of all CpG dinucleotides contained in the amplicon. PSQ is rather costly but it can be applied to heterogeneously methylated DNA and makes it possible to determine the methylation status of every single CpG dinucleotide in the amplicon. We therefore applied MS-HRM as a screening tool to select those amplicons requiring more detailed quantitative investigation and used PSQ to accurately determine the DNA methylation status of single CpGs. We found out that biotinylation of one of the primers, which is necessary for pyrosequencing, has no effect on the melting behavior of the DNA. Thus, PCR amplicons subjected to MS-HRM analysis can be directly used for quantitative DNA methylation analysis by pyrosequencing. Furthermore, we could show that by applying an advanced data processing procedure the average methylation status obtained by MS-HRM analysis is in accordance with the mean calculated from the methylation level obtained for single CpG dinucleotides by PSQ.