5'-CG-3' and 5'-CNG-3' sites frequency in microRNAs, %

Species	Content of GC in genome, %	Site 5'-CG-3' The mean per genome	Site 5'-CG-3' micro-RNA	Site 5'-CNG-3' Expected frequency of 5'-CNG-3' sites in a random sequence	Site 5'-CNG-3' micro-RNA
Homo sapiens	42.00	1.00	2.41	4.41	6.13
Mus musculus	42.20	1.00	2.46	4.45	6.82
Rattus norvegicus	43.90	1.20	2.22	4.82	6.71
The mean	-	-	2.39	-	6.49

frequency parameters of dinucleotides 5'-CG-3' and trinucleotides 5'-CNG-3' in the studied microRNA sequences are presented in the table.

These findings show that 5'-CG-3' and 5'-CNG-3' sites are discovered in microRNA sequences more often than they should be found in random sequence. This circumstance is evidence of an important biological purpose of 5'-CG-3' dinucleotides and 5'-CNG-3' trinucleotides in microRNA sequences. In our opinion, complexes of microRNA and Argonaute protein scan nucleotide sequence of DNA strands while RNA polymerase is untwisting DNA molecule during the transcription. Recognition and binding of complementary site in DNA by microRNA leads to recruiting of DNA methyltransferases that methylate de novo cytosine in 5'-CG-3' dinucleotides and 5'-CNG-3' trinucleotides of DNA, which appeared to be bound with similar sites in the microRNA sequence. Histone deacetylase and histone methyltransferase are also attracted to DNA site, which was recognized by microRNA. They delete active chromatin marks.

Allelic exclusion appears, in our opinion, as a result of initiation by microRNA of DNA methylation de novo of all but one alleles that exist in the cell. The predecessor of this microRNA is transcribed from the antiparallel allele chain. Alleles whose antiparallel chains are less actively read by RNA polymerase, which, as we suggest, in the process of transcribing, releases DNA from microRNA bound to it, are inactivated. However, the quantity of microRNA transcribed from only one allele is insufficient to overcome the level above which the repression process of this allele is initiated de novo.

The mechanisms of microRNA-directed DNA methylation that mediate in particular allelic exclusion and other effects of the gene dose probably appeared in the evolutionary process of the purpose of maintaining stability of the cell genome and of counteraction to the horizontal gene transfer. With the aid of microRNAs, they suppressed functioning of transposons and protected cells from the excessive copying of mobile genetic elements.

564 Poster

P53 potentiates PTEN-mediated inhibition of EGFR downstream signaling pathway by cetuximab in prostate cancer cells

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Background: Cetuximab (Erbitux®) is a chimeric monoclonal antibody, directed against the extracellular domain of EGFR. Its activity has been shown to depend on the functionality of PI3K/AKT and MAPK signalling pathways as well as apoptosis induction in cells. The aim of the present study consisted in evaluating the consequences of re-introducing P53 on the PTEN mediated inhibition of PI3K/AKT and MAPK signalling by cetuximab in P53-deleted prostate cancer cells.

Materiel and Methods: P53 and PTEN gene were transfected using polyethylenimine. Cetuximab cytotoxicity, alone or combined with gene transfer was evaluated using MTT assays. Apoptosis induction was evaluated by DNA fragmentation, active caspase-3 expression and pro-apoptotic BAX expression analyses. Variations in the functionality of PI3K/AKT and MAPK signaling pathways were determined from phosphoprotein expression analysis using phosphoprotein array assay and western blot analysis.

Results: P53 gene transfer was found to enhance pten-mediated cell growth inhibition and apoptosis induction by cetuximab. This effect was found to be mediated by restoral of signaling functionality with significant decrease in phospho-AKT (40% to 63%), phospho-GSK3β (38% to 72%), phospho-p70S6K (33% to 45%) and phospho-ERK1/2 (27% to 53%), basal expression with consequent significant increase in cell growth inhibition (20-40%), and apoptosis induction (11-25%).

Conclusion: These results show that in addition to PTEN mutation, P53 status could be predictive of cell response to cetuximab through the functional impact of these mutations on cell signaling. The data presented put forward the interest of the analysis of signaling phosphoprotein expression to evaluate the functionality of the signaling pathways implicated in the response to cetuximab.

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565 Poster

Functional re-differentiation of prostate cancer derived cell lines by the anti-tumoral drug Mycophenolic Acid (MPA)

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Mycophenolic Acid (MPA), is a reversible and non-competitive inhibitor of Inositine Monophosphate Dehydrogenase (IMPDH), key enzyme of guanosine nucleotide biosynthesis. MPA has been shown to have an anti-proliferative effect on prostate cancer derived cell lines PC-3 and DU145, as well as to induce their partial re-differentiation.

We focus on the effects of MPA on gene expression of key genes and markers of prostatic epithelium differentiation, using established prostate cancer derived cell lines.

We seek to assess the link between observed partial re-differentiation in vitro and the expression levels of the drug's known targets, metabolic clearance and epithelial differentiation markers.

METHODS: Prostate cancer derived cell lines (LNCaP, PC-3 and 22Rv1) were cultured in presence and absence of MPA. Quantitative RT-PCR was done on cDNA synthetised from total RNA extracts, using Gene Specific Primers.

We quantified mRNA levels of several key genes responsible for Inositine Monophosphate (IMP) homoeostasis, MPA metabolic clearance, or known prostatic epithelium markers. Immunoblots were done on the corresponding cellular extracts for validation.

RESULTS: GUSB, ACTB, UBC & TUBB were deemed the most adequate endogenous control genes.

The isoforms IMPDH1 and IMPDH2 were shown to be regulated differentially. Whereas expression of IMPDH type 2 is clearly increased by the pharmacological treatment, IMPDH type 1 is in most cases downregulated (PC-3 and 22Rv1) or stable (LNCaP). Guanosine synthesis salvage pathway (HPRT) is also stimulated in the presence of the drug.

UGT1A10, the gene responsible for clearance of MPA, is upregulated by the treatment.

PSA, absent in the PC-3 cell line, could be detected after treatment. Expression levels of this gene were strongly increased in the other cell types. These data were confirmed through immunoblots. Other epithelial markers studied (CD10, CD13, CD26) are often upregulated, but show a cell-type dependent response.

The observed effects were neutralised in the presence of guanosine during treatment.

CONCLUSIONS: In our model, MPA causes differential regulation of the IMPDH isoforms, and induces the expression of the guanosine synthesis salvage pathway (HPRT) and of the gene responsible for catabolic clearance of the drug (UGT1A10).

PSA is clearly upregulated in all cell lines studied, as are the other tested epithelial differentiation markers. This comforts the model that MPA induces functional re-differentiation of prostate cancer derived cell lines.

566 Poster

Apoptosis in oral squamous cell carcinoma

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Backgound: Squamous cell carcinoma (SCC) encompasses at least 90% of all oral malignancies. Oral cancer holds the eighth position in the cancer incidence ranking worldwide and oral squamous cell carcinoma (OSCC) implies quite significant mortality and morbidity rates, which motivates the search of factors with prognostic relevance in order to better tailor the individual management of OSCC patients. Apoptosis is a genetically programmed form of cell death, which primarily functions to eliminate senescent or altered cells that are useless or harmful for the multicellular organism. In contrast, aberrations of the apoptotic mechanisms that cause