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.

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

S. Bouali[1], A.S. Chretien[1], C. Ramacci[1], M. Rouyer[1], S. Marchal[2], T. Galenne[3], P. Becuwe[4], P. Juin[3], J.L. Merlin[1]
[1]CAV, Unité de Biologie des Tumeurs EA3452 Nancy Université, Vandoeuvre-les-Nancy, France; [2] CAV, CRAN UMR 7039 CNRS-UHP-INPL, Vandoeuvre-les-Nancy, France; [3] CHU de Nantes, Institut de Biologie INSERM U601, Nantes, France; [4] Faculté des sciences, Laboratoire de Biologie Cellulaire EA 3446 Nancy Université, Nancy, France

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.

Study supported by the French Ligue Contre le Cancer.

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

R. Ramirez Morales[1], V. Agrapart[1], C. Mencacci[1], C. Moretti[1], G. Frajese[1], G.V. Frajese[2]
[1]University of Rome "Tor Vergata", Department of Internal Medicine, Rome, Italy; [2] University of Cassino, Faculty of Motor Sciences and Health, Rome, Italy

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.

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Apoptosis in oral squamous cell carcinoma

C. Malheiros Coutinho Camillo[1], C. Pidorodeski Nagano[2], S.V. Lourenço[2], J.H. Fregnani[3], A. Lopes Carvalho[4], L.P. Kowalski[5], F.A. Soares[1]
[1]Hospital AC Camargo, Pathology, SÃO PAULO, Brazil; [2] Dental School University of São Paulo, General Pathology, SÃO PAULO, Brazil; [3] School of Medical Sciences of Santa Casa de São Paulo, Morphology, SÃO PAULO, Brazil; [4] Hospital do Câncer de Barretos, Head and Neck Surgery, SÃO PAULO, Brazil; [5] Hospital AC Camargo, Head and Neck Surgery, SÃO PAULO, Brazil

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

excessive or deficient programmed cell death have been linked to a wide array of pathologic conditions. Materials and Methods: In this study, using a Tissue Microarray (TMA) comprising 136 cases of OSCC, we have analyzed the immuno-expression of proteins that inhibit (Bcl-2, Bcl-x) or promote (Bid, Puma, Bad and cytochrome c) apoptosis. The results were quantitatively analyzed using an automated imaging system (ACIS III) which detects levels of hue, saturation and luminosity, converting this signal into a numerical density measurement that ranges from 0 to 256. These numerical results were divided into terciles and the following scores were attributed – (1) low expression; (2) median expression; and (3) high expression. Analysis of the association between apoptosis' proteins levels and the demographic and clinicopathological characteristics of the patients were performed by the Chi-square test. Disease-free survival and overall survival probabilities were calculated based on the Kaplan-Meier method. Results: High expression of Bad was associated with patients below 60 years (p=0.018) and with alcohol consumption (p=0.014). High expression of Bcl-x was associated with the presence of vascular embolization (p=0.043) and low expression of cytochrome c was associated with moderatelly/poorly differentiated tumors (p=0.025). Overall survival in ten years is statistically better in patients who presented median expression of Bcl-2 than in patients with low or high expression (p=0.022). Conclusions: Our data suggests that the expression of apoptotic molecules might be used as a prognostic indicator in oral squamous cell carcinoma. Supported by FAPESP 98/14335-2 and 07/50608-4.

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Molecular study of the differential TSPAN6, TSPAN15, TSPAN17, TSPAN18 and CD82 tetraspanins expression between fibromatosis and metastatic sarcomas

K.C. Carvalho[1], S.M. Marques[1], M. Maschietto[1], A.C. Simões[1], L.F.L. Reis[1], F.A. Soares[1], I.W. Cunha[1]
[1]Department of Anatomic Pathology, Hospital A. C. Camargo, São Paulo, Brazil

Background: Tetraspanins are abundantly expressed transmembrane proteins with at least 33 members in humans. Their structure span four times the citoplasmic membrane and possess the ability to associate each other and with a large number of different transmembrane proteins. Reduced expression of these proteins has been frequently reported in metastatic lesions. Although there are a lot of studies on expression of tetraspanins in solid tumors, theirs expression profile or the role of these proteins in soft tissue tumors (STT) is still unknown. Materials and Methods: The mRNA expression of CD82, TSPAN6, TSPAN15 and TSPAN17 was investigated in samples of STT (including fibromatosis, leiomyosarcoma, liposarcoma, synovial sarcoma, fibrosarcoma, pleomorphic sarcoma, and others). Bioinformatic tools were used to analyze microarray data of 102 STT cases. Results: Quantitative RT-PCR (qRT-PCR) for CD82, TSPAN6, TSPAN15 and TSPAN17 was performed as technical for 54 of the 102 STT. The entire array was classified according to STKE database and we looked for functional modules that might discriminate fibromatosis (benign fibroblastic tumor) and sarcomas (with metastatic potential). Linear correlations of expression levels of pairs of selected genes were evaluated in order to find alterations in fibromatosis and sarcomas samples. These changes could be indicative of involvement of these genes interaction in the mestastasis or malignant potential. Adhesion molecules, including tetraspanins and integrins, seem to be a promising functional module. Changes in linear correlation of genes pairs were observed in TSPAN15 in relation to TSPAN6, that showed a negative correlation in fibromatosis and positive in others sarcomas (p

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Natural and synthesized inhibitors of the MAP-kinase pathway - study of their efficiency in vitro

F. Saab[1,2], S. Routier[1], V. Bénéteau[1], J.-Y. Mérour[1], F. Schoentgen[2]
[1]Institut de Chimie Organique et Analytique (ICOA), UMR CNRS 6005, Université d'Orléans, Orléans, France (2)Centre de Biophysique Moléculaire (CBM), UPR CNRS 4301, Orléans, France

The protein called PEBP/RKIP is a natural inhibitor of the MAP-kinase pathway (Raf/MEK/ERK pathway) in cells [1]. It regulates Raf-1 and MEK activity by direct interaction with these two kinases [2]. Considering the implication of MAP-kinase pathway deregulation in numerous types of cancer [3], we have decided to study the interaction between PEBP/RKIP and Raf-1 in order to design and synthesize Raf-1 inhibitors based on new molecular data.

In a first time we have developed several tests to measure in vitro the activity of Raf-1 and the cascade Raf-1/MEK/ERK. Three methods are currently used to test Raf activity. Two of them, namely Phosphocellulose Filter Binding Assay (PFBA) and Scintillation Proximity Assay (SPA) are based on the measurement of the incorporation of radiolabeled phosphate in the substrate. The third method is based on homogenous Time Resolved-Fluorescence Resonance Energy Transfer (TR-FRET) (LANCE ultra technology, Perkin-Elmer).

Each assay techniques will be developed and we will dress a comparison between them. Moreover, the first results concerning the efficiency of some natural and synthesized inhibitors of Raf-1 will be presented.

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569 Poster

Expression of c-kit/SCF tyrosine kinase signaling pathway and apoptotic genes in ionomycin/PMA treated Jurkat cells

K. Stankov[1], G. Bogdanovic[2], L. Popovic[2], S. Popovic[3], S. Tauszig-Delamasure[4], P. Mehlen[4], N. Sylvius[5]
[1]Medical Faculty Novi Sad, Department of Biochemistry, Novi Sad, Serbia; [2] Institute of Oncology, Department of Oncology, Sremska Kamenica, Serbia; [3] Medical Faculty Novi Sad, Department of Haematology, Novi Sad, Serbia; [4] Centre Léon Bérard, Cancer and Development CNRS FRE 2870, Lyon, France; [5] CEA/Institut de Génomique, Centre National de Génotypage, Evry, France

The principal objective of our study was to test the regulation of gene expression of SCF/c-kit tyrosine kinase signaling pathway and the genes involved in apoptosis (Bcl-2, Bax and Bak), in ionomycin/PMA treated Jurkat cells.

By the means of quantitative real-time PCR, we measured the c-kit, SCF, Bcl-2, Bax and Bak expression levels in Jurkat cells RNA, extracted from ionomycin/PMA treated and non-treated cells. The expression data were normalized to the expression levels of four housekeeping genes and cDNA concentration.

Our results show that in the Jurkat cells, in the absence of exogenous SCF (c-kit ligand), ionomycin/PMA treatment down-regulates the expression c-kit receptor, and induces moderate up-regulation of pro-apoptotic (Bax and Bak) and pro-survival gene Bcl-2, thus showing that the down-regulation of the c-kit does not disturb the balance between pro- and anti-apoptotic Bcl-2 family genes.

According to our data, SCF has a very important role in the c-kit signaling pathway-mediated activation and proliferation of Jurkat cells.

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Expression of MDGA2 novel gene is downregulated in human tumors

C. De Juan Chocano[1], A. Díaz López[1], P. Iniesta Serrano[1], A. Morán Millán[1], C. Frías García[1], P. Ortega Esteban[1], T. Fernández Marcelo[1], A. Sánchez Pernaute[2], A. Torres García[2], M. Benito de las Heras[1]
[1]Facultad de Farmacia, Bioquímica y Biología Molecular II, Madrid, Spain; [2] Hospital Clínico San Carlos, Servicio Cirugía Digestiva, Madrid, Spain

Introduction and aim: Human MDGA2 (hMDGA1) (MAM domain containing glycosylphosphatidylinositol anchor-2) is homologous to the hMDGA1 gene, which has been identified and characterized in our laboratory in the last few years. MDGA2 gene has been mapped to 14q21 and is highly conserved among mammals. Expression analysis in normal human tissues revealed that MDGA2 is expressed as a 5 kb mRNA in heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas. Furthermore, three different transcripts of 5, 7 and 9 kb were detected in human fetal brain. A 5 kb mRNA was also detected in all the human primary tumors analysed (breast, ovary, uterus, lung, kidney, stomach and colon). The purpose of this study has been to analyse MDGA2 expression level in different tumor types, by Real Time Quantitative PCR. Patients and methods: Sixteen primary colorectal cancer tissues, twentythree endometrial cancer tissues and five primary non-small cell lung carcinomas, were obtained from patients who underwent surgery at San Carlos Hospital in Madrid (Spain). As control samples, a pool of eight-ten normal tissues from colon, endometrium or lung were used. MDGA2 expression was analysed in all these samples by Real Time quantitative PCR, using the TaqMan® Gene Expression System from Applied Biosystems. Results: Results have shown a significant down regulation of MDGA2 gene expression, as compared to normal tissues, in eight of sixteen colorectal tumors analysed (50%); fourthteen of twentythree endometrial carcinomas (61%) and five of five lung carcinomas analysed (100%). A similar downregulation of the MDGA1 homologous gene was also detected when MDGA1 gene expression was analysed in the same tumor tissues. Conclusion: Expression of MDGA2 is downregulated in human colorectal, endometrial and lung tumors.