proliferator-activated receptor gamma (PPARg). This nuclear receptor can be activated by natural ligands such as 15-deoxy-delta(12,14)-prostaglandin J2 (15d-PGJ(2)) as well as synthetic drugs such as thiazolidinediones. The treatment of human breast cancer cell lines with PPARg agonists is known to have antiproliferative effects but the role of PPARg activation in the process remains unclear.

We investigated the effects of four PPARg agonists, Rosiglitazone (RGZ), Ciglitazone (CGZ), Troglitazone (TGZ) and the natural agonist 15d-PGJ(2), on two hormone-dependent breast cancer cell lines, MCF-7 and ZR-75-1. ERalpha signalling pathway was studied using transfection and ERalpha was detected by western blotting and immunocytochemistry. In the two cell lines, TGZ, CGZ and 15d-PGJ(2) induced an inhibition of ERalpha signalling associated with the proteasomal degradation of ERalpha. ZR-75-1 cells were more sensitive than MCF-7 cells to these compounds. Treatments that induced ERalpha degradation inhibited cell proliferation after 24 h. In contrast, 24 h exposure to RGZ, the most potent activator of PPARg disrupted neither ERalpha signalling nor cell proliferation. 9-cis retinoic acid never potentiated the proteasomal degradation of ERalpha. PPARg antagonists (T0070907, BADGE and GW 9662) did not block the proteolysis of ERalpha in MCF-7 and ZR-75-1 cells treated with TGZ. ERalpha proteolysis still occurred in case of PPARg silencing as well as in case of treatment with the PPARg -inactive compound Delta2-TGZ.

Taken together, these results demonstrate a PPARg -independent mechanism. Studies are in progress to increase our understanding of the molecular mechanisms leading to ERalpha proteolysis. Thiazolidinedione derivatives able to trigger ERalpha degradation by a PPARg -independent pathway could be an interesting tool for breast cancer therapy.

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Estrogen receptor beta target genes in estrogen receptor alpha negative cells

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Estrogens exert their action through their receptors (ER) alpha and beta and regulate the expression of genes involved in cell proliferation and differentiation. In recent years, ER beta has been an important focus of researchers because of both its proliferative and antiproliferative properties in breast cancer. To understand the role of ER beta in breast cancer, it is necessary to identify its downstream target genes. To achieve this, a previously developed MDA MB 231-tet-on-ER beta1 cell model system was used. Cells were depleted of estrogens and treated with 2μg/ml doxycycline (DOX) for 48 hrs and then with either 10nM 17-β-estradiol (E2), 500nM of ICI182780 (ICI) or 4 hydroxy tamoxifen (4HT) for 24 hrs. Total RNA was isolated and candidate target gene expression was quantified by real time RT-PCR. Initially five candidate target genes, Trefoil Factor 1 (TFF1), Epidermal growth factor receptor (EGFR), Transforming growth factor (TGF) alpha, lipophilin B and erythroblastic leukemia viral oncogene homolog2 (erbB2) were measured in one clone. ER beta induction (+DOX) resulted in 56 ± 9 (mean ± SD, n= 3) fold increase in TFF1 mRNA and surprisingly, E2 treatment resulted in only a further 2 ± 0.17 fold increase over DOX alone treated cells. EGFR, lipophilin B, TGF alpha and erbB2 mRNA showed 1.7 ± 0.6, 4.6 ± 1.09, 3.2 ± 1.49 and 1.5 ± 0.45 fold increase, respectively, in response to DOX. E2 further increased the expression of EGFR by 1.5 ± 0.05 fold, lipophilin B by 1.8 ± 0.2 fold, TGF alpha by 1.5 ± 0.11 fold and erbB2 by 1.4 ± 0.36 fold. In medium containing 5% FBS, ICI and 4HT resulted in reduction of candidate gene mRNA levels to 16 ± 14.9 % and 13 ± 3 % for TFF1, 70 ± 31.9 % and 40 ± 23.6 % for EGFR, 20 ± 17 % and 20 ± 9 % for lipophilin B, 22 ± 4.1 % and 12 ± 4.2 % for TGF alpha, 20 ± 28 % and 50 ± 42 % for erbB2, respectively compared to untreated cells. After treatment with the transcription inhibitor actinomycin D, induction of all mRNA by DOX +/- E2 was inhibited. The data suggest that ER beta with and without E2 regulated target gene expression primarily at a transcriptional level.

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Transcription factor PAX3 isoforms differentially regulate expression of the proto-oncogene c-Met in vitro

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The paired box gene, PAX3 encodes a transcription factor involved in myogenesis, melanogenesis and neurogenesis and has significant roles in cell migration, proliferation and survival in early embryonic development. PAX3 has seven differently spliced isoforms. Studies by our group and others have shown these isoforms exhibit different transcriptional specificities, activities and functions.

The proto-oncogene c-Met is a well established target gene regulated by PAX3. HGF/Met signalling is involved in many aspects of embryogenesis and abnormal Met signalling has been implicated in both tumour development and progression. In particular, this signalling is important in promoting tumour cell invasion and metastasis. We investigated the relationship between PAX3 isoforms and the regulation of c-Met expression using RT-PCR and Western blotting in PAX3 isoforms transfectants of mouse melanocytes. It was found that the expression of c-Met was up-regulated in PAX3c and PAX3d transfectants, but was unaffected in PAX3e, PAX3g and PAX3h isoform transfectants. A luciferase assay revealed that only PAX3c and PAX3d isoforms were capable of activating the c-Met promoter in vitro; PAX3e, PAX3g, and PAX3h had no effect on the c-Met promoter. Thus, some PAX3 isoforms differentially affect c-Met expression through varied transactivations of c-Met promoter. Our results indicate the different roles of PAX3 isoforms in tumourigenesis.

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Cyclophilin A is located at the centrosome and functions in the maintenance of genome stability

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Cyclophilin A (cypA) belongs to the evolutionarily conserved peptidyl-prolyl isomerase (PPIase) family which includes the structurally distinct FK-506 binding proteins, the parvulins, and the recently identified PTPA. The PPIase family catalyse the cis-trans isomerisation of peptide bonds located to the N-terminus of proline residues in polypeptide chains thereby altering protein conformation. As such, PPIases are believed to play a role in protein folding and transport, however their true cellular function remains unclear.

In recent years the parvulin, pin1, has been shown to regulate the activity of important cell cycle proteins and thus exhibit both tumour promoting and tumour suppressor activity. These contradictory observations raise the possibility that pin1 can function as a tumour promoter or as a conditional tumour suppressor in a cell-type-selective manner [1].

Recently cypA was found to be overexpressed in pancreatic and lung cancer cells suggesting a possible role during tumourigenisis [2], however its function during tumour development and progression is unknown. In this study we have found that cypA is overexpressed in cells derived from a number of solid tumours including breast, prostate and cervix, and haematopoietic malignancies such as chronic myeloid leukaemia, Jurkat T lymphoma and HL-60 pro-myelotic leukaemia.

Investigation into a possible function of cypA has shown that it is localised at the centrosome in interphase haematopoietic cells. During mitosis, cypA concentrates at the spindle poles and migrates to the midbody during cytokinesis. Centrosomal localisation of cypA was confirmed by double staining of cells with anti-cypA and anti-γ-tubulin, an important component of the conserved γ-tubulin ring complex (γ-TuRC) that regulates microtubule function during mitosis. Merged images illustrate co-localisation of cypA and γ-tubulin suggesting a potential role for cypA during cell division. In support of this, it was found that leukaemia and lymphoma cells that do not express cypA undergo defective cell division and become multinucleated. Furthermore cypA-/- cells display a weakened mitotic spindle checkpoint in response to the microtubule-disrupting agents, taxol and nocodazole. Measurement of mitotic index using phosphorylated histone H3 has revealed that cells that lack cypA exit mitosis prematurely without completing cytokinesis and subsequently re-enter mitosis leading to the accumulation of cells with >4N. Collectively, this study strongly suggests a novel role of cypA in the maintenance of genome stability and provides valuable information that will help elucidate the precise role of PPIases during tumourigenesis.

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563 Poster

Hypothesis of mechanism of microRNA-directed DNA methylation and its evolutionary descent

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We examined microRNA sequences in order to identify possible mechanism of sequence-specific targeting of genes during the transcriptional silencing. Human, mouse and rat sequences of mature microRNAs - in all 1083 sequences - were extracted from database miRBase, http://microrna.sanger.ac.uk/. We detected that only 20.96% microRNA molecules did not contain any 5’-CG-3’ or 5’-CNG-3’ site. The

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

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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.

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)

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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.

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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