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

A. Arumugam[1], L. Murphy[1]
[1]Manitoba Institute of Cell Biology, Department of Biochemistry and Medical Genetics, Winnipeg, Canada

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

S. Kumar[1], M. Slevin[2], P. Kumar[2], Q. Wang[2]
[1]Manchester University, Pathological Department School of Medicine, Manchester, United Kingdom; [2] Manchester Metropolitan University, School of Biology Chemistry and Health Sciences, Manchester, United Kingdom

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

J.H. Bannon[1], F.T. Bane[1], M.M. Mc Gee[1]
[1]Conway Research Institute, School of Biomolecular and Biomedical Science, Dublin, Ireland

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

V. Halytskiy[1]
[1]Palladin Institute of Biochemistry of NAS Ukraine, Molecular Immunology Department, Kiev, Ukraine

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