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Luciano di Croce (Spain) • Jessica Downs (UK)

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Programme Book

We show that HOTAIR is down-regulated in ARMSp primary samples and cell lines compared to their respective controls. Conversely, it is up-regulated in fusion-negative ERMS and ARMSn.

The microRNA miR-196a follows a similar trend. Both pri-miR-196a-2, co-linear with HOTAIR, and pri-miR-196a-1, located on the 17q21 region, are up-regulated in ERMS/ARMSn and down-regulated in ARMSp. HOTAIR and miR-196a expression are also associated to those of HOXC10 and HOXC11.

Interestingly, ARMSp cells treated with 5-azacytidine show a time-dependent induction of HOTAIR whereas pri-miR-196a-2 is not over-expressed.

In conclusion, results from these preliminary experiments indicate that the expression of genes on the chromosome 12 region surrounding HOTAIR and miR-196a could be de-regulated in RMS. They also suggest a different regulation of the involved genes in the two RMS subtypes since HOTAIR can be silenced by methylation in PAX3-FOXO1 RMS cells. Future experiments will shed light on the role of HOTAIR in this soft tissue sarcoma.

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Axolotl Oocyte Extracts Mediate Epigenetic Reversion of Breast Cancer Malignant Phenotype by Blocking the Cell Cycle

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Axolotl oocyte extracts (AOE) can reprogram the breast cancer cell epigenome by modifying DNA methylation and histone modifications at the promoter region of silenced tumour suppressor genes, resulting in loss of cell tumorigenicity. This study aimed to investigate signalling pathways involved in an attempt to identify specific extract molecules responsible for the reprogramming of breast cancer cells. Microarray analysis of reprogrammed tumours showed a cell cycle block that was confirmed by BrdU staining of xenograft tumours. The phenotype was recapitulated in 2D and 3D matrigel cultures after 4 days of reprogramming, with reprogrammed cells showing a significant decreased entry in S phase of the cell cycle. After reprogramming, decreased expression of JUN and up-regulation of CDKN1B (p27) was measured. This was associated with a decrease in phosphorylation of p38MAPK and p44/42 MAPK signalling pathways, but not of JNK and TGFβ, thus suggesting a possible role of these pathways in the cell cycle block. CDKN1B protein also showed a nuclear localisation in reprogrammed tumour xenografts compared to controls. At a genome-wide level, the reprogramming of breast cancer cells is associated with a decrease in H3K9me2 and H3K9me3 and in increase in H3K27me3 and H3K9Ac. No significant change in H3K4me3 levels was observed. The pattern of changes was generally maintained at the time point of injection into xenografts (12 hours after reprogramming). Histone modifiers inhibitors were next used to dissect the correlation between epigenetic modifications and cell signalling during reprogramming. The histone acetyltransferases inhibitor garcinol did not have any effect on the observed decrease in MAPK activity, whereas the histone demethylase inhibitor tranylcypromine was able to block this effect. These results suggest that axolotl oocytes contain complex activities that may cooperate to remodel the epigenetic and signalling landscape of breast cancer cells leading to reversion of malignancy.

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Stable DNA methylation in early phases of epithelial-to-mesenchymal transition induced by mesenchymal stromal cells in vitro

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Epithelial-to-mesenchymal transition (EMT) significantly affects the risk of metastasising in breast cancer. Plasticity and reversibility of these processes suggest that epigenetic mechanisms could be their key drivers. To study changes in global and gene specific DNA methylation accompanying EMT process, we induced EMT by mesenchymal stromal cells (MSCs). The therapeutic potential of unmodified gold nanoparticles (AuNPs) mediated by their proposed ability to reverse EMT and their possible epigenetic toxicity was also investigated.

Methods: Indirect co-cultivation of human breast cancer SK-BR-3 cells in conditioned medium from adipose tissue-derived (AT) MSCs was used for induction of EMT. To reverse EMT, 20 nm size gold nanoparticles (AuNPs) were applied. DNA methylation of LINE-1 sequences and promoter methylation of *TIMP3*, *ADAM23* and *BRMS1* genes were evaluated by pyrosequencing.

Results: Despite the presence of EMT-associated morphological and gene expression changes, EMT induced by AT-MSCs had almost no effect on DNA methylation. Although treatment for 24, 48 or 72 hours with AuNPs at a concentration of 3 μg/ml slightly decreased gene expression of EMT-associated markers, it did not alter global or gene-specific DNA methylation.

Conclusions: Our results suggest that changes in DNA methylation are not detectable *in vitro* in early phases of EMT. Previously published positive findings could represent rather the sustained presence of potent EMT-inducing signals or the synergistic effect of various epigenetic mechanisms. Treatment with AuNPs slightly attenuated EMT, and their therapeutic potential needs to be further investigated.

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The Use of Highly Validated Rabbit Monoclonal Antibodies to Analyze Epigenetic Marks and Mechanisms in Disease.

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Research in the field of epigenetics has grown at a rapid pace since the discovery of the first histone acetyltransferase enzymes 19 years ago. Since then, significant advances have been made in our understanding of the basic epigenetic mechanisms regulating gene expression and genomic stability, and the impact of epigenetic deregulation on cancer, inflammation, metabolism, and neurological diseases. Much of our knowledge of these mechanisms comes from the utilization of antibodies to probe the protein levels and localization of transcription factors, chromatin regulators and histone modifications in different cell and tissue types, and across the genomes of a multitude of organisms. While antibodies have been a key reagent driving advancements in epigenetic research, there are increasing numbers of publications raising concerns about the quality of the antibodies being used in biomedical research. A very concerning large number of antibodies, both commercially available and those developed by individual laboratories, have not been completely validated, some showing a lack of specificity and sensitivity even in western blot or dot blot assays. Many additional antibodies show specificity in these assays, but fail to work in more demanding assays such as immunofluorescence (IF), flow cytometry (Flow), immunohistochemistry (IHC) and chromatin IP (ChIP). Even high quality, well-validated polyclonal antibodies have issues with reproducibility, as antibody attributes often change from lot to lot. Recent advancements in antibody technologies, specifically the development of rabbit monoclonal technologies presents solutions to many of these problems. We will demonstrate how the utilization of rabbit monoclonal technology combined with thorough antibody validation can lead to generation of high quality rabbit monoclonal antibodies that show exquisite specificity, sensitivity, and reproducibility across multiple applications, including IF, IHC, flow, ChIP and ChIP-Seq.

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The Cancer-Associated K27M Mutation in variant Histone H3.3 abrogates Cellular Senescence.

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Histone variants (such as the histone H3 variant H3.3) play important roles in a multitude of cellular processes. We have shown previously that disruption of the H3.3 predisposition complex Daxx/ATRX by the PML-RAR oncoprotein leads to block of senescence, effectively immortalizing cells (Korf et al. PNAS 2014). Cancer-associated mutations within the H3.3 gene have been reported in paediatric Glioblastoma (Schwarzentruber et al, Nature 2012). We have expressed the cancer–associated H3.3 K27M mutant in primary murine bone marrow by retroviral transduction, to analyze its effect on senescence, which these cells enter after 6-8 (1:5) passages *ex vivo*. To our surprise, expression of the H3.3 K27M onco-mutant not only led to a polyclonal immortalization of the murine bone marrow, but also produced cells with a highly undifferentiated phenotype. We conclude from these observations that assignment of H3.3 plays a pivotal role in cellular senescence and differentiation.

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Paracrine signals from tumor cells are involved in modulation of microRNA-signature of stromal fibroblasts in PDAC

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Pancreatic adenocarcinoma (PDAC) is characterized by a strong desmoplastic reaction. The most abundant stromal cell type, cancer-associated fibroblast (CAF), has primarily been linked with tumor-promoting characteristics. CAFs have diverse origins and are epigenetically and functionally distinct from resident normal fibroblasts (NFs). A newly described concept of cell-cell-communication comprises the exchange of complex signals via 30-80nm-sized membrane vesicles, called exosomes. Here, we study the effects of tumor cell-secreted exosomes on resident fibroblasts.

AsPC1 was utilized as a model for human PDAC. Primary cultures of NF and CAF were obtained from an orthotopic xenograft model of AsPC1 in CD1/nude mice. Exosomes were purified from conditioned medium using OptiPrep™ density gradient (Van Deun et al., J Extracell Vesicles, 3, 2014). GeneChip® miRNA 4.0 microarrays and qRT-PCR were applied for identification of microRNA-signatures. Modified Boyden chambers were used for indirect co-culture (3µm pores) and transmigration assays (8µm pores).

We found 89% of mature microRNAs that were expressed by AsPC1 cells to be secreted in AsPC1 exosomes. However, the microRNA-composition of cells and exosomes differed, revealing a more profound microRNA-reduction than -enrichment in exosomes (ratio 1.7:1.0). A set of 12 microRNAs was highly expressed in AsPC1 exosomes and also significantly upregulated in CAF compared to NF (FC≥2, ANOVA p≤0.05, FDR p≤0.25). These comprised four members of the miR-200 family (miR-200a, -200b, 200c, -141), which is known to regulate EMT and proliferation in tumor cells. Indirect co-culture of NF with AsPC1 was sufficient to emulate the same changes in microRNA-expression and correlated with a 92%±38% (Student's ttest p=0.001; n=5) enhanced motility of fibroblasts.

This data suggests that exosomal shuffling of microRNAs from tumor cells to surrounding fibroblasts may be involved in remodeling of resident fibroblasts into a CAF-like status.