O-9 LOSS OF CSMD1 EXPRESSION DISRUPTS CELL MORPHOLOGY AND MAMMARY DUCT FORMATION WHILE ENHANCING PROLIFERATION, MIGRATION AND INVASION

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CUB and Sushi multiple domains protein 1 (CSMD1) maps to 8p23, a region deleted in many cancers, and thought to be a tumour suppressor gene. Loss of CSMD1 expression is associated with reduced survival in breast cancer patients. CSMD1's function is unknown; however, CSMD1's structure suggests it is involved in signal transduction. Here, we have investigated the function of CSMD1. CSMD1 expression was silenced in MCF10A, MDA-MB-435 and LNCaP cell lines by shRNA and functional assays were performed.

Loss of CSMD1 expression disrupted cell morphology and caused 30% ($p < 0.001$), 32% ($p = 0.03$) and 56% ($p < 0.001$) increase in cell proliferation of MDA-MB-435, LNCaP and MCF10A, respectively, compared to controls. Also MDA-MB-435 and MCF10A shCSMD1 cells showed reduced adhesion to matrigel (32%, $p = 0.0005$ and 44%, $p = 0.0006$, respectively), and to fibronectin (39%, $p = 0.004$ and 32%, $p < 0.001$, respectively). Moreover, loss of CSMD1 expression enhanced cell migration of MDA-MB-435 and MCF10A and caused 33% ($p < 0.001$) increase in cell invasion of MCF10A, compared to control. The MCF10A 3D model revealed that loss of CSMD1 expression resulted in the development of larger poorly differentiated breast acini and impaired lumen formation.

Loss of CSMD1 expression induced behaviour consistent with cellular transformation. Our data supports the concept that CSMD1 participates in signaling pathways that regulate a range of key cellular processes involved in the suppression of a transformed phenotype.

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O-10 HIGH TIMM17A EXPRESSION IS ASSOCIATED WITH POOR CLINICAL OUTCOME AND UNFAVOURABLE PATHOLOGICAL PARAMETERS IN HUMAN BREAST CANCER

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Introduction: Mitochondrial dysfunction can be associated with genomic instability and has been implicated in the pathogenesis of breast cancer (BC). The mitochondrial protein, Translocase of Inner Mitochondrial Membrane 17 homolog A (TIMM17A) contributes to a pre-protein import complex, essential for mitochondrial function. In this study, TIMM17A mRNA expression was evaluated in benign and malignant breast tissues and correlated with pathological and clinical outcomes.

Methods: BC tissues ($n = 127$) and normal tissues ($n = 33$) underwent RNA extraction and reverse transcription, transcript levels were determined using real-time quantitative PCR and normalized against CK19. Transcript levels were compared and then analysed against tumour size, tumour grade, oestrogen receptor (ER) status, nodal involvement, TNM stage, Nottingham Prognostic Index (NPI) and clinical outcome over a 10 year follow-up period.

Results: Compared to normal tissue, TIMM17A mRNA expression was higher in BC ($p = 0.006$), TNM-1 ($p = 0.05$), TNM-2 ($p = 0.034$), NPI-2 ($p = 0.041$), patients with progressive disease ($p = 0.017$) and those who died from BC ($p = 0.026$). Expression increased with tumour grade; grade 1 versus 2 ($p = 0.007$), grade 1 versus 3 ($p = 0.065$, NS) and grade 1 versus 2 and 3 ($p = 0.0048$). Higher transcript levels were associated with ER-α positivity ($p = 0.073$, NS) and ER-β negativity ($p = 0.015$). Nodal positivity was significantly associated with higher transcript levels ($p = 0.046$). Compared to disease free patients, TIMM17A expression was significantly higher in those with progressive disease and patients who died of BC ($p = 0.037$). Higher transcript levels were significantly associated with poorer overall survival after a median follow-up of 10 years ($p = 0.010$). TIMM17A expression emerged as a strong independent predictor of overall survival in multivariate analysis ($p = 0.033$).

Conclusion: TIMM17A mRNA expression is significantly associated with unfavourable pathological parameters including tumour grade, nodal positivity, TNM stage and NPI; in addition to adverse clinical outcomes such as progressive disease and overall survival. TIMM17A offers utility as a prognostic marker and a novel mitochondrial target for potential therapeutic strategies.

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O-11 INHIBITING DNA METHYLATION AND HISTONE DEACETYLATION ENHANCES RESPONSE TO DOCETAXEL IN BREAST CANCER CELLS

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Introduction: Understanding the mechanisms of drug resistance is important to improve and deliver effective therapy. Epigenetic modifications like DNA methylation and histone deacetylation can alter gene expression, due to gene silencing, and may represent mechanisms of drug resistance.

Methods: Breast cancer cells (MCF-7 and MDA-MB-231), and their docetaxel-resistant sublines, were treated with either trichostatin A (TSA), 5-aza-2′-deoxycytidine (decitabine) or in combination. DNA methyltransferase activity and global methylation were measured by ELISA-based assays, and histone acetylation levels were measured by western blot. Response to docetaxel of cells treated with inhibitors was measured using cell viability assay. Gene expression analysis was performed using a microarray-based quantitative PCR system. Western analysis was used to validate gene expression changes at the protein level.

Results: Docetaxel resistance was associated with changes in DNA methyltransferase activity and global methylation. Treatment with decitabine alone did not alter response to docetaxel. In contrast, TSA enhanced docetaxel sensitivity in MCF-7 cells whereas MDA-MB-231 cells were unaffected. Combination

treatment with decitabine and TSA increased docetaxel sensitivity in MCF-7 and MDA-MB-231 cells ($P < 0.05$). An array of 84 genes identified 5 genes decreased in resistant cells whose expression was upregulated after decitabine and TSA treatment ($P < 0.05$). Western analysis confirmed expression changes in only one gene, SERPINE1, in docetaxel-resistant cells.

Conclusions: Docetaxel resistance is associated with changes in the DNA methylation machinery. Inhibiting DNA methylation and histone deacetylation, in combination, overcomes resistance of breast cancer cells to docetaxel. Our findings indicate that decreased SERPINE1 expression is associated with docetaxel resistance.

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O-12 TRANSLATIONAL EXPLORATION OF PIK3/Akt pathway activation in early invasive breast cancer

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The phosphatidylinositol 3-kinase (PI3K)/Akt pathway is a frequently deregulated pathway in breast cancer (BC). Akt arguably relays a plethora of extracellular signals to modulate diverse biologic effects, including cell proliferation, growth, motility, and survival, downstream of PI3K activation. However, complexity and diversity in the upstream/downstream arms of this pathway challenge considerably the clinical evolvement of effective therapies.

This study aims to study expressions of PIK3CA and phospho-Akt1 (pAkt) in BC, with respect to proteins upstream/downstream of Akt activation, clinicopathologic parameters, and disease outcome. PIK3CA and pAkt (ser473) were evaluated by immunohistochemistry on tissue microarrays containing 1202 early invasive BC with long term follow-up.

In this study, pAkt overexpression was associated with patients' age, estrogen and androgen receptors, cytokeratin (CK)18, CK19 and PTEN expression. Loss of pAkt was correlated with higher grade, CK5/6, p53 and Ki-67 labelling index. Luminal-like tumours displayed more pAkt positivity than triple negative/basal-like subtypes. However, pAkt overexpression was not associated with breast cancer-specific (BCSS) or metastasis-free survival (MFS). Four combinatorial phenotypes were identified based on PIK3CA and pAkt expression, with considerable proportions being $PIK3CA^{-}/pAkt^{+}$ or $PIK3CA^{+}/pAkt^{-}$. These phenotypes were significantly associated with BCSS ($p = 0.001$) and MFS ($p = 0.002$).

Although pAKT is an oncogene that correlated with poor prognostic variables, it was not a prognostic marker. Combinatorial phenotypic groups of PIK3CA/pAkt denoted, at translational level, functional complexity within the upstream and downstream network of Akt activation with significant impact on patients' outcome. These findings may help developing adequate therapeutic regimens against specific components of this key signalling pathway.

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O-13 *D-gLUCURONYL C5-EPIMERASE* INHIBITS BREAST CANCER CELLS PROLIFERATION THROUGH THE TUMOUR SUPPRESSOR GENES ACTIVATION

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D-Glucuronyl C5-epimerase (GLCE) is one of the key enzymes of the biosynthesis of heparan sulphate proteoglycans. Down-regulation of *GLCE* expression in human breast tumours and cancer cell lines suggested a possible involvement of the gene in breast carcinogenesis. To test the hypothesis, we ectopically expressed GLCE in breast cancer cells MCF7 and showed that re-expression of *D-glucuronyl C5-epimerase* significantly inhibited proliferative activity of MCF7 cells according to CyQUANT NF Cell Proliferation Assay and did not affect the viability of the cells in Colony Formation Test. The antimitotic effect of *D-glucuronyl C5-epimerase* in human breast cancer cells probably is realised via the activation of tumour suppressor genes SYK (+8.1-fold), BRCA1 (+3.5-fold), p53 (+3.3-fold) and E2F1 (+3.00-fold) and change of a balance of pro- and anti-apoptotic factors BCL2 (+4.2-fold), NFKB1 (+2.6-fold) and TNF (+4.6-fold) (PathFinder RT Profiler PCR Array). Also, *GLCE* re-expression in MCF7 cells considerably changed expression of some genes involved in angiogenesis (IL8, IFNB1, TNF and TGFB1) and invasion/metastasis (SYK, NME1, S100A4) suggesting a possible antimetastatic effect of *GLCE in vivo*.

In summary, the ability of the *D-glucuronyl C5-epimerase* to suppress proliferation of breast cancer cells through the affecting different key genes involved in cell cycle regulation, angiogenesis and invasion/metastasis molecular pathways supposes the gene as a new potential candidate for diagnosis and treatment of breast cancer.

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O-14 EXPRESSION AND ACTIVATION OF Akt AND NFkB IN BREAST CANCER PATIENTS

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Background: It is postulated that Akt activates the Nf-kB pathway to promote tumour growth and survival in breast cancer cells.

Material and methods: Tissue microarray technology was employed to analyse tissue from 426 breast cancer patients. Immunohistochemistry was performed using antibodies for pAkt (phosphorylated at serine 473), NF- kB and pNFkappaB (phosphorylated at serine 536). Expression was assessed using the weighted histoscore method by two independent scorers.

Results: Median age was 62 years, median tumour size was 20 mm, 48% were pathologically graded G2 and 31% G3 and 48% were lymph node positive. Ninety-eight patients had unilateral