

### 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 normal-

ized 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- $\alpha$  positivity ( $p = 0.073$ , NS) and ER- $\beta$  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