Calcium signalling in epithelial-mesenchymal transition induced by hypoxia in breast cancer cells

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Hypoxia is a hallmark of the cancer microenvironment and induces epithelial-mesenchymal transition (EMT) in breast cancer cells. EMT is a process whereby cancer cells acquire a more invasive phenotype. We have recently characterized the remodelling of calcium (Ca^{2+}) signalling as a consequence of EMT induced by epidermal growth factor (EGF). In this study we aimed to elucidate the remodelling of Ca^{2+} signalling in a model of EMT induced by hypoxia in MDA-MB-468 breast cancer cells.

The induction of EMT by hypoxia (1% O_2) was confirmed *via* protein level of vimentin (after 48 h hypoxia) and mRNA levels of some of the main EMT markers including vimentin, Snail, Twist, N-cadherin and the ratio of CD44/CD24 (after 24 h hypoxia). The mRNA levels of fifty Ca^{2+} pumps, channels, sensors and G-coupled receptors in the presence and absence of hypoxia (24 h) were evaluated using real time RT-PCR. This led to identification of four specific targets that were significantly up-regulated in hypoxia compared to normoxia (21% O_2). P2Y6 purinergic receptor, as one of these up-regulated targets, showed a three-fold increase in mRNA levels by hypoxia. Selective pharmacological inhibition of P2Y6 significantly reduced cellular migration of the mesenchymal like MDA-MB-231 breast cancer cell line. Gene expression profiling of 458 human breast tumours showed elevated levels of P2Y6 in basal breast cancer subtypes compared to Luminal A and Luminal B subtypes. Furthermore, breast cancer patients with high P2Y6 levels (n = 651) showed reduced overall survival rates compared to patients with low levels of P2Y6 (n = 464) ($P = 0.019$).

In conclusion this study suggests that hypoxia-induced EMT is associated with alterations in Ca^{2+} signalling and P2Y6 purinergic receptor expression. Further studies may identify a specific Ca^{2+} pump, channel or receptor that may represent a target for the mesenchymal phenotype of breast cancer cells and a potential therapeutic strategy for the control of breast cancer metastasis.