438 Neuronal calcium sensor-1 (NCS-1) in the regulation of calcium homeostasis and cell death in MDA-MB-231 basal breast cancer cells

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Background: Altered calcium (Ca^{2+}) signalling in cancer cells may promote cancer hallmarks such as resistance to apoptosis. Proteins regulating these signals represent attractive therapeutic targets. Neuronal calcium sensor-1 (NCS-1) is associated with tumour aggression and poor prognosis in breast cancer patients. However, the characterisation of NCS-1 in breast cancer molecular subtypes, the effects of NCS-1 silencing on intracellular Ca^{2+} homeostasis in breast cancer cells and on the cytotoxic effect of the anti-cancer drug doxorubicin, remain unexplored.

Aim: To assess the expression of NCS-1 in public breast cancer datasets and assess the consequences of silencing NCS-1 on intracellular Ca^{2+} signaling and sensitivity to doxorubicin in the MDA-MB-231 basal breast cancer cell line.

Methods: The expression of NCS-1 in patient breast tumours was stratified by PAM50 molecular subtype and assessed using breast cancer public datasets. MDA-MB-231 cells stably expressing the GCaMP6m Ca^{2+} sensor were transfected with non-targeting control or NCS-1 siRNA. The effects of NCS-1 silencing on cytosolic Ca^{2+} in response to Ca^{2+}-mobilising agonists (ATP, trypsin and cyclopiazonic acid (CPA)) and on constitutive Ca^{2+} influx were measured using a Fluorescent Imaging Plate Reader (FLIPR). The sensitivity to doxorubicin (24 h) following gene silencing of NCS-1 was determined by propidium iodide staining.

Results: NCS-1 was expressed higher in basal molecular subtype breast cancers. Silencing NCS-1 did not alter cytosolic Ca^{2+} changes induced by ATP, trypsin or CPA treatment. However, NCS-1 silencing suppressed constitutive Ca^{2+} influx. NCS-1 silencing also promoted MDA-MB-231 cell death in combination with doxorubicin (1 μM) treatment.

Discussion: These results implicate NCS-1 in basal breast cancer, a subtype with poor prognosis. Indirect modulators of endoplasmic reticulum Ca^{2+} levels such as NCS-1 may alter constitutive Ca^{2+} influx pathways and influence processes important in cancer such as sensitivity to anti-cancer agents.

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439 Understanding the physiological role of endogenous allosteric modulators in the muscarinic acetylcholine receptors

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Introduction. Allosteric binding sites on G protein-coupled receptor (GPCR) can be targeted by synthetic or natural (endogenous) molecules (van der Westhuizen et al., 2015). However, the (patho)physiological role(s) of many endogenous allosteric modulators remain poorly understood. One interesting example is major basic protein (MBP), a highly basic peptide that acts as a negative allosteric modulator (NAM) of acetylcholine (ACh) at airway M_2 muscarinic acetylcholine receptors (mAChR; Jacoby et al., 1993). We hypothesized that, in addition to MBP, other endogenous basic peptides, including the antimicrobial, LL-37, involved in chemotaxis, maturation of immune cells and apoptosis (Kahlenberg et al., 2013) could also interact allosterically with the M_2 mAChRs and have major physiological impacts.

Aims. To characterise the pharmacological properties and the putative (patho)physiological roles of LL-37 at mAChRs.

Methods. Using IMR-32, a native cell line endogenously expressing human M_2 mAChRs and mouse tissues predominantly expressing mouse M_2 mAChRs, we performed [^{3}H]-NM-