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**Project title:** Preclinical identification of local anaesthetics that target colon cancer cells
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**Background:**
Several studies suggest that the general anaesthesia (GA) and opioid-sparing effects of regional nerve block during tumour excision reduce cancer recurrence. GA and opioids inhibit immune function; a reduced requirement may contribute to the apparent role of nerve block in reducing recurrence and metastasis. In addition, *in vitro* studies suggest that local anaesthetics (LAs) may directly suppress the metastatic potential and self-seeding of cancer cells through inhibition of their voltage-activated $Na^+$ channels (VASCs). This raises the possibility of an additional therapeutic benefit of LAs delivered systemically and/or directly onto the tumour during excision (see Baptista-Hon et al., 2014, BJA).

Metastatic breast and colon cancer cells express a neonatal variant of the $Na_V1.5$ VASC not found in normal adult tissue. This project will identify LAs and related compounds that target these VASCs at concentrations that spare cardiac $Na_V1.5$. The neonatal $Na_V1.5$ channel has novel functional properties that may promote inhibition by some LAs. We will screen several LAs to identify those with the highest selectivity for VASCs on colon cancer cells. The potencies of LAs as neonatal $Na_V1.5$ inhibitors will be correlated with their potencies as inhibitors of cell motility and invasion (see Baptista-Hon et al., 2014, BJA). This preclinical study will be used to guide subsequent clinical trials investigating the benefit of intraperitoneal LA administration during colorectal cancer surgery.

**Methods:**
Aim 1. *Characterise VASCs on metastatic colon cancer cells*. Metastatic colon cancer SW620 and HT29 cell lines express $Na_V1.5$ channels. They also express mRNA for the putative auxiliary proteins: $\beta1$, $\beta3$ and scotin. We will characterise the composition of the $Na_V1.5$ channel complex involved in colon cancer cell invasion. We will take several complementary approaches to this: i) Immunoprecipitation ($Na_V1.5$ antibody) and western blot analysis (with antibodies to auxiliary subunits proteins) to examine which VASC complexes assemble in the membranes of colon cancer cells. ii) siRNAs to knockdown candidate proteins and examine the effects on a) the expression of functional VASCs using the whole-cell patch-clamp technique to characterise $Na^+$ currents in SW620 cells and b) the ability of cancer cells to invade through Matrigel. (iii) We will test the functional consequences of expressing recombinant adult and neonatal $Na_V1.5$ channels with auxiliary proteins in HEK cells to emulate the properties of VASCs in SW620 cells.

Aim 2. *Determine the concentration-dependence for state-dependent inhibition of VASCs on cancer cells and cardiac myocytes by LAs*. By plotting activation and inactivation curves in the presence of different concentrations of each of the LAs we will determine their potencies for shifting the steady-state current. The current of greatest importance is that observed at the resting membrane potential for the colon cancer cells (-40 mV). We will also examine the proportion of steady-state current remaining at this potential directly. We will quantify the relative use dependence of current block and the rate of reversal from blockade at differing membrane potentials. A key parameter when considering the rationale for delivering LAs during surgery onto the tumour prior to excision is the rate of recovery from block. Our preliminary observations suggest that recovery from block at -40 mV is extremely slow and may exceed the time for synthesis of new $Na_V1.5$ channels. We will investigate this by treating colon cancer cells with LAs in culture and examining several hours of wash off. We anticipate that the inhibitory actions will last for several hours and recovery will be dependent on new VASC synthesis and therefore preventable by application of cyclohexamide.

Aim 3. *Establish the concentration-dependence of inhibition of motility and invasion by LAs*. We will use the Matrigel invasion assay to examine invasion through 8 μm pores. Disposable Boyden chambers are used for this purpose. Those with pores but no Matrigel are used to establish cell motility, while those containing Matrigel provide an assay of matrix degradation and invasion. We will establish the concentration dependences for the LAs and related drugs as inhibitors of motility and invasion. We have used this approach previously to examine the suppression of SW620 cell invasion by lidocaine and TTX.

**Results:**

We have made good progress on all three aims.

Aim 1: We have successfully co-immunoprecipitated scotin with $Na_V1.5$ (Fig. 1A). These data support the functional analysis which indicates that scotin alters the biophysical properties of $Na_V1.5$ VASCs (Fig. 1B). We will make progress on the siRNA knock-down approach in the next 12 months.

Aim 2: We have charaterised state-dependent blockade of $Na_V1.5$ blockade by ropivacaine (Baptista-Hon et al., 2014, BJA), lidocaine (Fig. 1C) and levo-bupivacaine (Fig. 1D). We are writing a manuscript which will include the levo-bupivacaine and lidocaine data. The latter are the subject of a recently submitted Anaesthesia Research Society abstract (Maltman et al., 2014, ARS).

Aim 3: We have completed this aim for lidocaine (Fig. 1E) and ropivacaine (Baptista-Hon et al., 2014, BJA; Maltman et al., 2014, ARS). We will investigate flecainide and amitriptyline in the next 12 months.

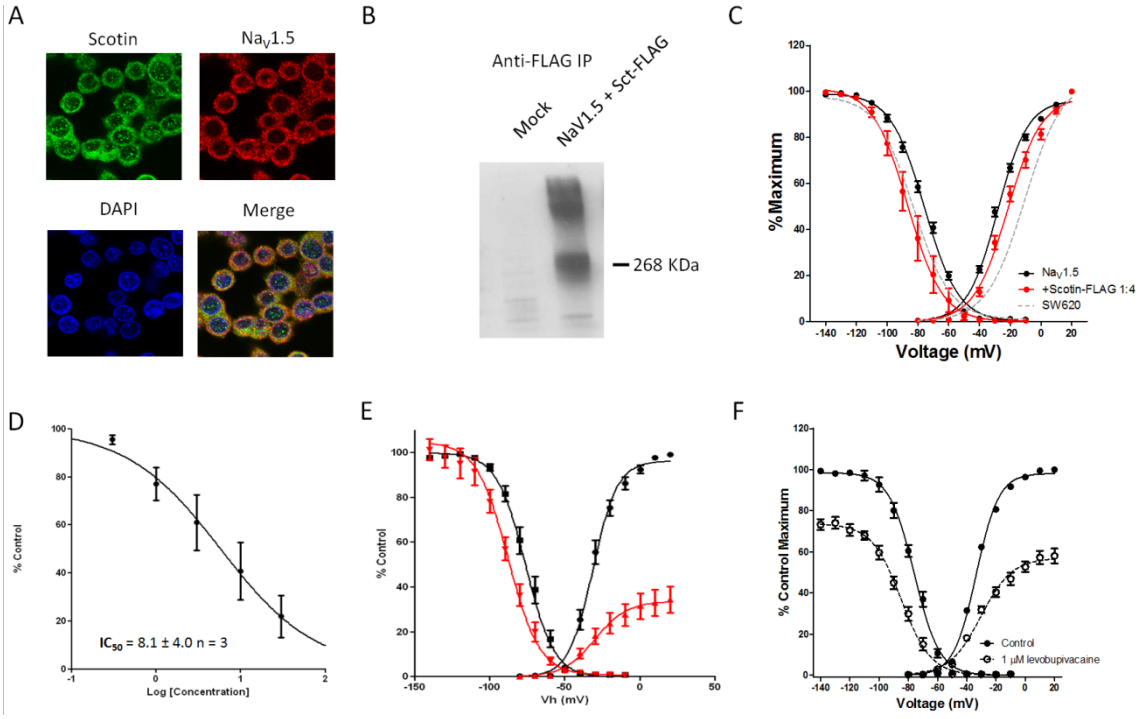


**Figure 1.** A, Co-localisation of scotin and $Na_V1.5$ in SW620 cells. B, Scotin-FLAG pull-down with FLAG antibody, followed by western analysis with anti-$Na_V1.5$ antibody reveals co-immunoprecipitation. C, Scotin alters the voltage-dependence of $Na_V1.5$ activation and inactivation when both proteins are expressed in HEK cells. Taken together A-C demonstrates that scotin acts as an NaV1.5 auxiliary subunit. D, Lidocaine inhibits NaV1.5 with high potency (recordings at -80 mV). E, Lidocaine (10 μM) shifts inactivation to more hyperpolarized potentials, but does not inhibit current at a potentials between -140 and 110 mV at which there is negligible inactivation. Blockade of NaV1.5 by lidocaine is therefore inactivation-state dependent. F, Levo-bupivacaine (10 μM) inhibits at all potentials. And therefore causes state-independent block.

**Publications:**

Baptista-Hon DT, Robertson FM, Robertson GB, Owen SJ, Rogers GW, Lydon EL, Lee NH, Hales TG. Potent inhibition by ropivacaine of metastatic colon cancer SW620 cell invasion and $Na_V1.5$ channel function. Br J Anaesth. 2014 113 S: i39-i48.

Maltman I, Elajnef T, Baptista-Hon DT, Weir C, Hales TG. High potency inactivation-dependent blockade of $Na_V1.5$ channels by lidocaine allows inhibition of colon cancer cell invasion without affecting phasic $Na^+$ currents. Anaesthesia Research Society Abstract. Oct 2014.