Characterization of voltage-gated sodium channel subtypes expressed in the human rhabdomyosarcoma cell line TE671

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Introduction: The TE671 cell line has been widely used as a model of human medulloblastoma[1]. Further analysis showed that its phenotypic and cytogenetic properties were similar to the human muscle RD cell line, hence, TE671 was ascribed as rhabdomyosarcoma rather than medulloblastoma[2]. Previous studies showed that TE671 cells exhibited voltage-gating properties, cation selectivity and neurotoxin sensitivity-characteristic of 'classical' sodium channels of excitable cell[2].

Method: This study further characterizes the voltage-gated sodium channel (VGSC) subtypes expressed in the TE671 cell line via: (1) Gene expression of the VGSCs determined using qPCR to measure RNA levels based on two reference genes, *β-actin* and *GAPDH*, (2) Electrophysiology studies where membrane currents were recorded under voltage clamp conditions using the patch clamp technique in whole-cell configurations.

Results: The expression pattern shows that $Na_V1.7$ is expressed ~100-fold more than $Na_V1.4$ and ~1000-fold more than any other VGSC subtypes (Fig. 1). The VGSCs expressed in TE671 cells display kinetic and voltage-activation characteristics resembling VGSCs expressed in many other cells. Macroscopic sodium currents display a typical transient time course with a rapid rise to a peak followed by an exponential decay. The rates of early activation and subsequent inactivation accelerate and approach a maximum in response to test potential, of greater depolarization. The magnitude of peak sodium current increased from negligible values at V_t = -40 mV and reached a maximum at V_t= ~0 mV. The half-maximal activation ($V_{1/2}$) was 22.7±0.8 mV with a slope (κ) of 8.46±0.71 mV, while the voltage-dependence of fast inactivation of the current was 64.2±0.7 mV (Fig 2). The VGSC of TE671 cells fall into the Tetrodotoxin (TTX)-sensitive category; TTX IC_{50} recorded was 24.3 nM. The VGSC current was also potently blocked by the $Na_V1.7$-selective spider toxin, HWTX-IV, with an IC_{50} of 8.27 nM (Fig 3).

Conclusion: Although we predicted that the muscle type $Na_V1.4$ may be the channel expressed in TE671 cells, the present study suggests that the $Na_V1.7$ subtype is predominantly expressed as shown in both the gene expression and electrophysiology studies (indicated by high sensitivity to the $Na_V1.7$ selective, HWTX-IV). This elevated the importance of the TE671 cell line as a model for pain studies.

References:

1. Stratton *et al.* (1989). *Carcinogenesis* **10(5)**: 899-905.
2. Gambale *et al.* (1990). *Molecular Brain Research* **7**: 123-129.

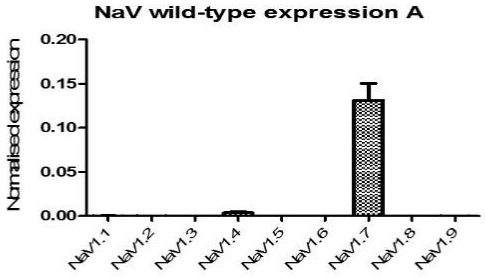

Fig 1. Normalised expression of the nine VGSCs genes, relative to *β-actin* and *GADPH*, used as endogenous controls (mean ± SD, n=3) (normalised using the comparative cycle threshold method). The expression pattern clearly shows that $Na_V1.7$ is expressed ~100-fold more than $Na_V1.4$ and ~1000-fold more than any of the other VGSCs genes.

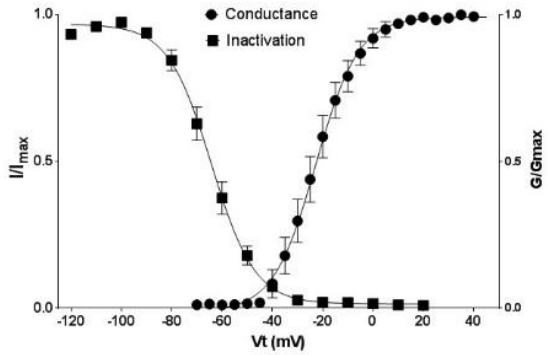

Fig 2. Voltage-dependence of activation and inactivation of TE761 cells, $V_{50.act}$ = −22.73 ± 0.792 mV with a slope (κ) = 8.46 ± 0.707, $V_{50.inact}$ = −64.23 ± 0.7421 mV (mean ± SEM, n=20).

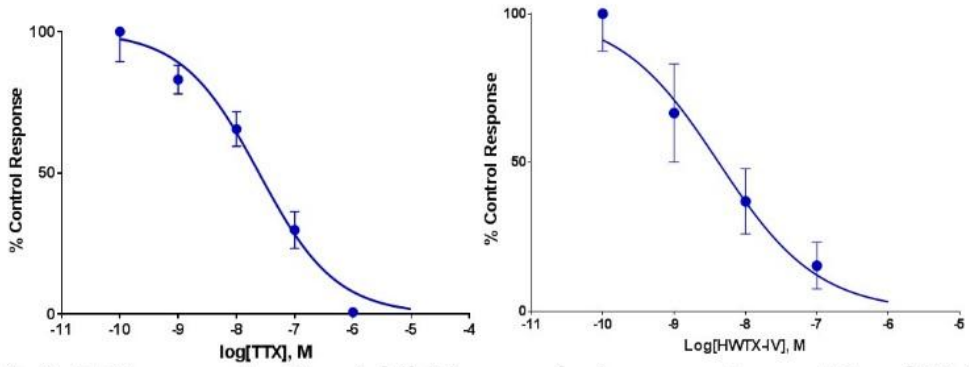

Fig 3. TTX concentration-inhibition analysis generates an IC_{50} of 24.33 nM. HWTX-IV; concentration-inhibition analysis generates an IC_{50} of 8.27 nM.