Development of phenotypic *in vitro* models of pain in early drug discovery

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Background

Neuropathic pain is caused by a direct damage or by a disease affecting central or peripheral nervous system (Treede et al. 2008). Despite of the high prevalence of the pathology, the development of a treatment for neuropathic pain is still an unresolved issue. This is due to the fact that drugs approved for the treatment of the disease such as pregabalin or gabapentin show low efficacy and several serious adverse effects (Woolf & Mannion. 1999). Since the late 20th century, drug discovery programs have consisted of target-based assays, evaluating the effect of a molecule over a previously identified target. Therefore, new analgesics share similar mechanisms of action and suffer lack of translationality. Thus, target-independent phenotypic assays could represent a progress for the development of new analgesics for the pathology (Lee & Berg. 2013).

Dorsal root ganglion (DRG) neurons are the cells involved in the transmission of painful stimuli from the limbs to the central nervous system (Chen et al. 2007). There are several immortalized DRG sensory neural cell lines such as F11 and ND7/23 cells, which are hybrids of DRG neurons and neuroblastoma cells. These cells could constitute suitable *in vitro* models for early drug discovery.

Hypothesis & Aims

The employment of immortalized DRG sensory neural cell lines would provide enough throughput, assay sensitivity and reproducibility for HTS. These cellular phenotypic models could allow for the screening of novel analgesics with innovative mechanisms of action or combinations of synergic mechanisms of action that in a conventional approximation would not have been possible to detect. The objectives of our study are:

1. Evaluate the best conditions of differentiation in order to prompt the development of neuronal structures.
2. Assess the sensitivity of differentiated neurons to painful stimuli exposing them to ATP and KCl and quantifying Dynamic Mass Redistribution (DMR) signal.

Materials & Methods

CELL GROWTH

Cells were grown in high glucose DMEM supplemented with 10% non-dialyzed foetal calf serum, 100U/ml penicillin/ 0.1mg/ml streptomycin and 2mM glutamine. For the differentiation, the same medium supplemented with differentiation factors was employed.

CELLULAR DIFFERENTIATION ASSESSMENT

Cells were seeded in 384 well plates previously treated with poly-D-lysine and laminin. Cells were grown at 37ºC in a 5% CO_2 humidified atmosphere. After 24h, growth medium was replaced by differentiation medium and cells were differentiated for 72h until the experiment was performed. Three differentiation protocols were tested for each cell type. Before staining, cells were fixed with 3% paraformaldehyde in PBS and were blocked with a buffer containing 2% BSA and 0.1% Triton X100. A 1:400 dilution of anti-TrkA receptor rabbit antibody in blocking buffer was added overnight. In the morning, cells were incubated with 1:300 anti-rabbit antibody marked with CFL647 and 1:3000 Hoechst® 33342 nucleic acid stain in blocking buffer. Photographs were taken using an Operetta High Content Imaging System® (Perkin-Elmer). Fluorescence was quantified by Harmony High Content Imaging and Analysis® software (Perkin-Elmer) and an unpaired t-test was performed using GraphPad Prism® (GraphPad Software INC.).

DYNAMIC MASS REDISTRIBUTION (DMR) ASSAY

Cells were seeded in 384 well plates at a density of 10000 cells per well and, after 24h of growth, cells were exposed to differentiation medium. This medium consisted of growth medium complemented with 0.5mM dibutyryl cAMP and 30µM forskolin (F11 cells) or 1mM dibutyryl cAMP, 60µM forskolin and 1.3ng/mL NGF (ND7/23 cells). In both cases, medium was supplemented with 0.5% dialyzed FBS. DMR assays were performed as previously described (Schröder et al. 2011).

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- Lee & Berg. J. Biomol. Screen 2013, 18(13):1143-55.
- Schröder et al. Nat. Protoc. 2011, 6(11):1748-60.
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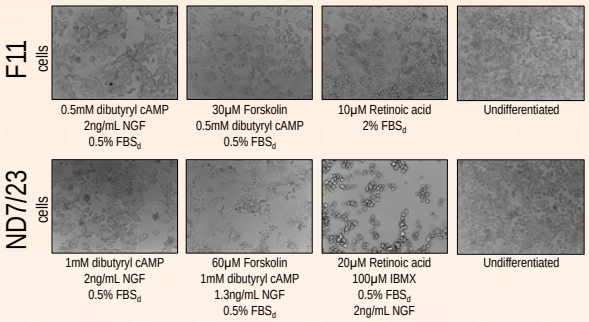
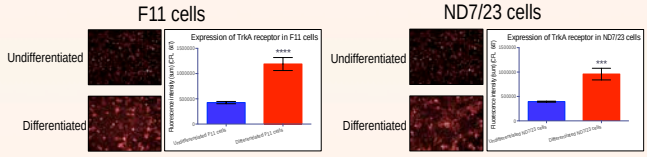
Results

The use of forskolin and dibutyryl cAMP as differentiation factors led to the acquisition of neural DRG phenotype.



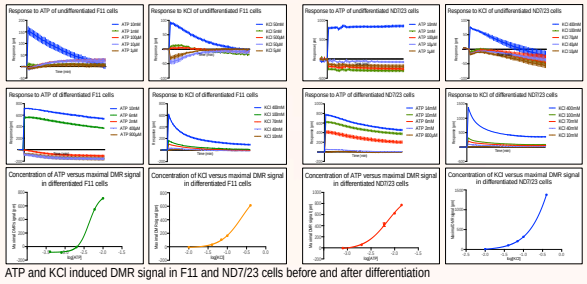
Brightfield photomicrographs of F11 and ND7/23 cells, before and after differentiation, employing three different differentiation methods.

The use of forskolin and dibutyryl cAMP as differentiation factors led to the expression of neuronal DRG markers.



Fluorescence photomicrographs of F11 and ND7/23 cells and expression of TrkA receptor in both cell lines, before and after differentiation process employing forskolin and dibutyryl cAMP as differentiation factors.

Differentiated cells respond to algogenic stimulus in label-free DMR assays.



ATP and KCl induced DMR signal in F11 and ND7/23 cells before and after differentiation

Potency (EC_{50}) and efficacy (Emax) of the algogens KCl and ATP in both cell lines (F11 and ND7/23) after differentiation.

	F11 cells		ND7/23 cells	
	ATP	KCl	ATP	KCl
EC_{50} (M)	$4 \cdot 10^{-3}$	≈ 0.2	$7 \cdot 10^{-3}$	≈ 0.4
E_{max} (pm)	769	892	1004	1378

Conclusions

1. The treatment with 30µM forskolin, 0.5mM dibutyryl cAMP and 0.5% FBS_d in F11 cells, and with 60µM forskolin, 1mM dibutyryl cAMP, 1.3ng/mL NGF and 0.5% FBS_d in ND7/23 cells, for three days, prompted differentiation of both cell lines to cells with DRG neuron phenotype.
2. Both differentiated cell lines responded to ATP and KCl in a concentration-dependent manner in the label-free assay, obtaining with ATP an EC50 of $4 \cdot 10^{-3}$M and $7 \cdot 10^{-3}$M in F11 and ND7/23 cells, respectively. In the case of KCl, F11 and ND7/23 cells responded with an EC50 of 0.2M and 0.4M, respectively.

These differentiated cell lines could represent new biological tools for establishing novel phenotypic in vitro models for HTS of drugs to treat neuropathic pain.

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