G-protein Coupled Receptor Expression Patterns Are Altered as Human Embryonic Stem Cell Derived Neural Cultures Differentiate

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INTRODUCTION

Human embryonic stem (hES) cells and their progeny can provide a novel tissue source for understanding developmental pathways, pharmaceutical screening and tissue replacement therapies. G-protein coupled receptors (GPCRs) comprise the largest cell-surface receptor superfamily and are the largest class of drug targets. The study of GPCR signaling in hES cells allows signaling mechanisms to be studied in endogenously expressed receptors in non-transformed cells. We characterized GPCR transcript expression in three cellular populations at different developmental stages: WAO9 human embryonic stem cells, Wa09 derived neural progenitor cells (ENSTEM-A™, Millipore) and differentiated human neural cultures maintained 1 week in culture (Hn2™ cells, ArunA Biomedical). Here we examine:

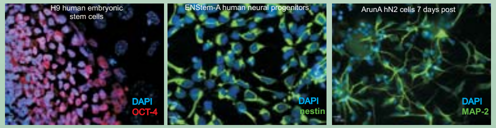
① Relative abundance of GPCR transcipts within and between cell populations

② Relative up-regulaiton of transcripts between cell populations

③ Physiological responses to GPCR activation

Our goal: To characterize GPCR transcript expression in human hES cell derived neural tissue

METHODS



Cell Populations Used: H9 human embryonic stem cells, Enstem™ neural progenitors (Millipore), hN2™ human neural cells (ArunA Biomedical). hN2 cells are derived from hNP cells by culturing hNPs for two weeks in the absence of bFGF. hN2 cells send out neurite projections within hours of plating. hN2 cells used for this study were plated for 1 week prior to harvest for RNA isolation.

Cell Culture: WA09 (WiCel) hES cells were cultured in DMEM/F12, L-glutamine, MEM nonessential amino acids, penicillin, streptomycin, bFGF and 20% KSR on mitotically inactivated murine embryonic fibroblasts. Neural progenitors were cultured in neurobasal media supplemented with penicillin/streptomycin, L-glutamine, B27, bFGF and LIF. hN2 cells were grown in hNP medium without bFGF.

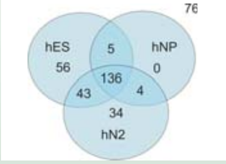
Immunohistochemistry: Cells were fixed in 2% paraformaldehyde and stained using standard Immunofluorescence protocols. Antibodies against the following proteins used were: nestin (1:200, Neuromics), oct4 (1:200,Santa Cruz), MAP-2 (1:500, Millipore).

Ca++ Imaging: Cells were plated into 96 well plates, assays were run on a Flexstation 3 (Molecular Devices) plate reader using a FLIPR calcium 4 assay kit (Molecular devices).

Real time PCR: Real time PCR was run on an Applied Biosystems 7900HT system. Gene expression data (3 replications) were acquired and SDS software was used to estimate relative fold change values using ΔΔCt quantification method. 18s rRNA was used as an endogenous control and either hNP or hES cells were used as a normalizer sample.

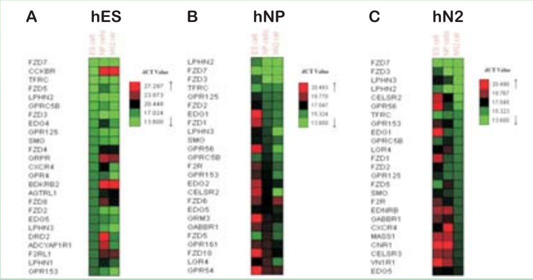
① RELATIVE ABUNDANCE OF GPCR TRANSCRIPTS

Distribution of detectable transcripts for three cell populations



The distribution of detectable transcripts for three cell populations: hES cells expressed the widest array of detectible GPCR transcripts and the hNP population the most restricted.

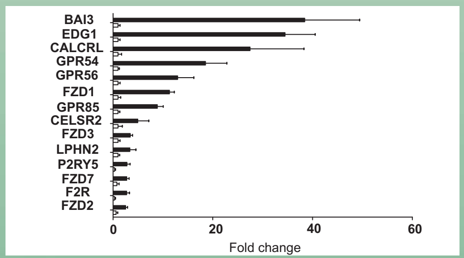
Relative abundance of GPCR transcripts



The 25 most abundant transcripts (lowest ΔCT) ranked in order for hESC (A), hNP (B), and (C) hN2 cells. Transcripts involved with WNT signaling (FZD1-8) were highly expressed in all populations, lysophosphatidic acid signaling (EDG 2,4) in hES and hNP cells, and sphingosine-1-phosphate (EDG 1,5) in hN2 cells.

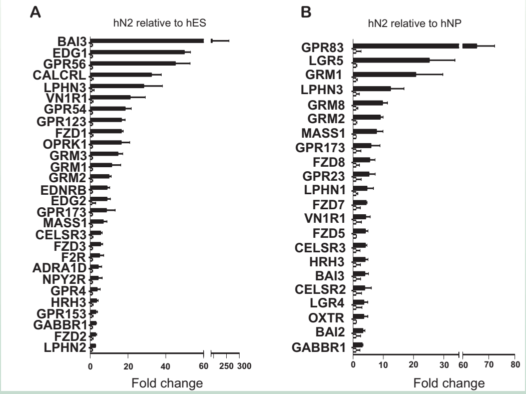
② UP-REGULATION OF GPCR TRANSCRIPTS

Up-regulated expression of GPCR transcripts in hNP relative to hES cells



Significantly (p< 05) up-regulated GPCR transcripts in hNPs relative to hESCs. GPCRs highly up-regulated in neural progenitors are those linked to brain angiogenesis, (BAI3,EDG1,CALCRL,F2R), proliferation and neurogenesis(EDG1,GPR54,P2RY5,GPR85), cell adhesion (GPR56,CELSR2), wnt signalling (FZD1,3,7,2) and neurite extension (CELSR2).

Up-regulated expression of GPCR transcripts in hN2 cells

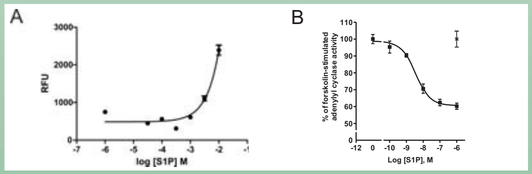


(A) Significantly (p<05) up-regulated GPCR transcripts in hN2s relative to hES. In addition to a subset of transcripts up-regulated by hNPs, hN2 cells up-regulate GPCRs related to neurotransmission (OPRK1; GRM1,2,3; ADRA1D;NPY2R;HRH3; GABBR1), sensory perception (VN1R1;MASS1; GPR4), neurite retraction (CELSR3), proliferation (EDG2,EDNRB) and orphan receptors expressed by the CNS (GPR123;173).

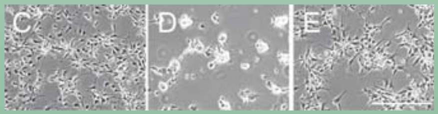
(B) Significantly (p<05) up-regulated GPCR transcripts in hN2s relative to hNP. In addition to a subset of transcripts identified in (A) GPCRs up-regulated are involved with glucocorticoid signaling (GPR83), neurotransmission (GRIM8;OXTR) and wnt signaling (FZD8).

③ PHYSIOLOGICAL RESPONSES TO GPCR ACTIVATION

Intracellular signaling response in hNP in to sphingosine-1-phoshate



Effects of sphingosine-1-phosphate on hN2 culture morphology



(A-B) EDG1, a gene transcript for the sphingosine-1-phosphate (S12P) receptor was highly expressed in hNP and hN2 cells. (A) Internal calcium signal [Ca^{++}], in hNPs increased in response to S1P consistent with expression of functional EDG1 receptors. (B) hNP cells were treated with 50 µM forskolin and various concentrations of S1P for 20'. (* = in the presence of Ptx).

(C-D) hN2 cells were plated for 24 hours and monitored by time lapse microscopy (B). 2 hours following addition of 1 µM S1P resulted in neurite retraction and a clustering of the cells (C). One hour post-washout of S1P the hN2 cells were spreading out extended neurites thus identifying S1P as a strong mediator of neurite retraction in human neural cells. Bar = 250 µM.

CONCLUSIONS

hES cells displayed the widest array of GPCR transcripts, while neural progenitors displayed the most restricted population.

The Frizzled (FZD) family of receptors were among the most abundantly expressed transcripts across all populations.

Neural progentitors upregulated GPCR transcripts important to brain angiogenesis, cell proliferation, neurogenesis, and cell adhesion.

Further differentiated hN2 cells displayed up-regulation of a wider population of transcripts including GPCRs involved with neurotransmission.

Functional assays demonstrated responses to sphingosine-1-phosphate in both hNP and hN2 populations of cells.

hES cells and their derived tissue provide a unique model to study endogenous GPCR signaling in non-transformed cells for drug screening applications and to further our understanding of GPCRs role in developmental pathways.

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