A Role for Glucocorticoid Receptor in Retinal Stem Cells

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Introduction

- Synthetic glucocorticoids (sGC) such as betamethasone (Beta) and dexamethasone (Dex) are administered to women at risk for preterm birth to enhance fetal lung maturation, reducing the risk of respiratory distress, interventricular hemorrhage necrotizing enterocolitis and retinopathy of prematurity (ROP).[1]
- Studies in animal models and humans indicate that exposure to sGCs has harmful effects on neural development, cognition, and behavior.[2]
- We have previously shown that prenatal Dex exposure alters neural stem cell (NSC) proliferation, neuronal differentiation and adult behavior in mice.
- RNAseq studies of cerebral cortical NSCs identified genes that are dysregulated in response to Dex exposure.

Hypothesis

- Several of the Dex regulated genes identified in mouse cerebral cortical NSCs have been implicated in eye disorders, suggesting that sGC exposure may impact eye development.
- For example, antenatal Dex exposure is associated with a decreased severity of ROP by downregulating genes implicated in neovascularization.[9]
- These findings lead to the hypothesis that prenatal sGC exposure will lead to gene expression changes in the visual system that may impact the long term biology of the eye.

Methodology

- To validate the changes in gene expression obtained by RNAseq, cerebral cortical NSCs were cultured from E14.5 mouse embryos until passage 2.
- Dex, or Beta, or Ethanol (control), all at 10^{-7}M, were administered and incubated for 4 hours.
- Subsequently, RNA was isolated using Trizol per manufacturer's instructions, converted to cDNA, and run in a quantitative Polymerase Chain Reaction (qPCR) with gene specific primers for our genes of interest using SYBR Green detection kit (Sigma-Aldrich).
- GAPDH acts as an internal control for RNA and cDNA quality since sGCs do not affect its expression.
- Using the delta delta CT method, we compared fold change to evaluate how gene expression is affected in the cells treated with sGC compared to the control.
- Retinas were isolated from E14.5 embryonic eyes by mechanical dissection, triturated into single cells and seeded in Stem Cell Inc. Proliferation Media. Retinospheres formed after 7 days and were passaged to P2 for qPCR analysis.
- Affymetrix Gene Chip expression profiles were compared using their transcriptome analysis console (TAC, Fischer Scientific). Samples were analyzed using a T-Test and significance assigned at 0.05.

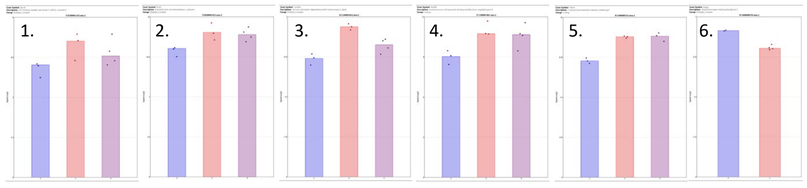
Results

Table 1. RNAseq identifies sex specific Dex induced differences in gene expression in several genes implicated in visual and neurological disorders

Gene	Sex	FC	p-value	Sex Difference	Basal Difference	T-Test p-value	Associated Pathology/Function
GPR179	Female	2.89	3.97E-05				Congenital Stationary Night
GPR179	Male	4.92	2.38E-09	Yes	No	0.22	Blindness, retinal disorders[3]
CYP1B1	Female	1.99	3.54E-02				
CYP1B1	Male	4.71	7.79E-06	Yes	No	0.13	Primary Congenital Glaucoma[4]
TSC22D3	Female	5.57	1.07E-29				Inhibits pro-inflammatory
TSC22D3	Male	4.68	1.12E-24	No	No	0.90	molecules[5]
NEDD9	Female	3.49	1.82E-11				Cell Cycle Regulation and
NEDD9	Male	4.07	7.37E-14	No	No	0.31	Cancer Metastasis[5]
EFHC1, PAQR8	Female	2.49	1.16E-05				
EFHC1, PAQR8	Male	3.78	3.73E-10	No	No	0.24	Juvenile Myoclonic Epilepsy[6]
BCAT1	Female	3.65	5.79E-32				
BCAT1	Male	3.65	5.32E-32	No	No	0.62	Glioblastoma[7]
CAMKK1	Female	3.58	7.77E-12				Regulates apoptosis and
CAMKK1	Male	3.41	6.56E-11	No	No	0.28	promotes cell survival[5]
ABCA4	Female	5.69	2.11E-11				
ABCA4	Male	3.38	8.43E-07	Yes	No	0.36	Macular Degeneration[8]
VSTM4	Female	5.37	8.32E-16				L-VGCC currents in retinal
VSTM4	Male	8.10	9.06E-22	Yes	No	0.17	photoreceptors[5]
COL6A3	Female	16.02	1.42E-08				
COL6A3	Male	28.78	3.63E-06	Yes	Yes	0.02	Dystonia, Bethlem Myopathy[5]

Table 1: Summary of RNAseq results showing significant difference in Dex regulated genes in males and females with associated disorders.

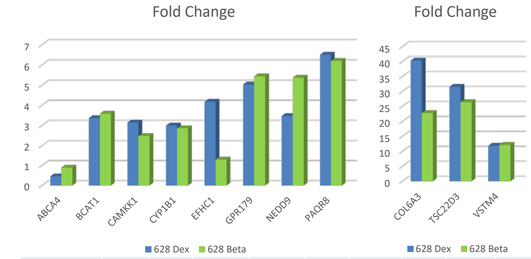
Figure 1. Affymetrix data showing Dex and Beta log2 fold changes for select gene targets



1. ABCA4 - ATP-Binding Cassette (ABC) Transporters
2. BCAT1 - Branched Chain Amino Acid Transaminase 1
3. CAMKK1 - Calcium/Calmodulin Dependent Protein Kinase Kinase 1
4. NEDD9 - Neural Precursor Cell Expressed Developmentally Down-Regulated Protein 9
5. VSTM4 - V-Set And Transmembrane Domain-Containing Protein 4
6. IMPG2 - Interphotoreceptor Matrix Proteoglycan 2

Figure 1: Clinically, Beta is predominantly used prenatally while Dex is predominantly used postnatally. To determine if the genes of interest were differentially regulated by Dex vs Beta, Affymetrix Dex versus Beta gene expression array were examined. Mutations in IMPG2 have been implicated in retinitis pigmentosa.[10] IMPG2 was not included in the results from RNAseq (table 1) but was later included when a statistically significant difference between Ethanol and Dex regulation was found in the Affymetrix data.

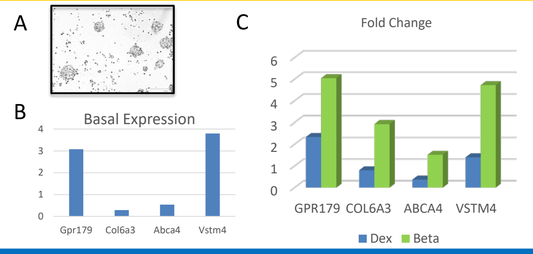
Figure 2: Validating candidate genes from RNAseq data via qPCR in cerebral cortical NSCs and comparing Dex and Beta differences



Fold Change	ABCA4	BCAT1	CAMKK1	CYP1B1	EFHC1	GPR179	NEDD9	PAQR8	COL6A3	TSC22D3	VSTM4
628 Dex	0.45	3.36	3.14	2.99	4.17	5.03	3.46	6.50	40.30	31.51	11.84
628 Beta	0.89	3.58	2.47	2.85	1.29	5.43	5.36	6.19	22.74	26.36	12.12

Several of the genes identified by RNAseq were validated in independent samples. Significant differences in fold induction in response to Dex or Beta are shown.

Figure 3: Retinal stem cell cultures (RSCs) were established from E14.5 eyes. Select candidate visual genes identified from RNAseq data in Table 1 were validated by qPCR in RSC cultures.



To determine if the sGC induced "visual" genes in the cortex were expressed in the retina and are responsive to sGC. Retinal stem cell cultures were established (A), exposed to sGC for 4 hours and qPCR performed with gene specific primers.
Basal gene expression (B) and Dex versus Beta induced fold change are shown (C).

Conclusion

- The expression of our 11 candidate eye genes are altered by Dex and Beta in cortical NSCs.
- A select number of genes are also expressed in retinal stem cells and are induced by Dex and Beta.
- In both cortical and retinal stem cells, Dex and Beta induce differential effects on target gene expression.
- VSTM4 is an L-type voltage gated calcium channel, implicated in stem cell proliferation, that is upregulated by Dex or Beta. ABCA4 is a retinal specific membrane ABC transporter and its compromised transporter activity result in degeneration of photoreceptors.
- Similarly GPR179, a member of the G-protein coupled receptor family, has been shown to be involved in synaptic transmission between light excited rod cells and on-bipolar neurons. Disturbance in this process results in night blindness and retinal dysfunction. In our results, Dex and Beta both differentially upregulated its expression in NSCs and RSCs. Therefore, sGC might be involved in modulation of synaptic transmission.
- The findings from these experiments can be used in targeted gene therapy for premature infants who receive sGC treatment and may be at risk of developing ophthalmological problems.

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