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ESTABLISHMENT OF CONDITIONAL TRANSGENIC WNT5A EMBRYONIC STEM CELL LINE

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ABSTRACT

Wnt5a activity is believed to be stage-dependent during neural differentiation process of mouse embryonic stem (ES) cells. As an effort to unravel the role of Wnt5a during the process, we aimed to establish a transgenic mouse ES cell line carrying a conditional Wnt5a construct using a *Cre/loxP*-based system. We managed to establish the transgenic line where the expression of Wnt5a transgene is temporally controlled upon exposure to a non-detrimental dosage of 4'-hydroxytamoxifen (4-OHT) for 48 hours. The system allows us to closely monitor the effects of overexpressing Wnt5a at specific time points during neural differentiation process on the formation of neurons, particularly the dopaminergic neurons. The conditional Wnt5a transgenic lines are hoped to shed light in generating a high homogeneous population of dopaminergic neurons suitable for cell-replacement therapy for Parkinson's disease.

1.0 Introduction

Generating pure populations of defined neuronal subtypes from embryonic stem (ES) cells remains a challenge as little is known about the genes that govern ES cell differentiation. Using transgenic mouse ES cell line, we aim to uncover the mechanisms that regulate neural differentiation of ES cells, especially towards the generation of midbrain dopaminergic (DA) neurons, by focusing on roles played by Wnt family genes. Wnt proteins are a large family (19 members in mammals) of signaling molecules that have been implicated in the regulation of a diverse set of developmental processes. Many studies have implicated one particular Wnt, *Wnt5a* in promoting dopaminergic neurogenesis in the midbrain (1,2). We have observed a dynamic expression profile of Wnt5a during neural differentiation process of ES cells (3), suggesting its activity during the process to be stage-dependent with higher expression of the gene detected during the late stage or the neuronal stage. Combining two techniques, *Cre/loxP*-based genetic recombination and ligand-dependent activation of Cre, we propose to monitor the effects of Wnt5a activity at specific time points using our newly established conditional transgenic Wnt5a embryonic stem cell line. This system offers a great advantage in examining the effects of overexpressing Wnt5a at specific time points during the neural differentiation process as an effort to generate highly homogenous functional DA neurons *in vitro.*

2.0 Materials and Methods

2.1 The construction of pCAG-floxed-*neo*pA-*Wnt5a* plasmid

In generating the plasmid, a 2.5 kb of *Wnt5a* cDNA was excised from pCMV.SPORT6 (Mammalian Gene Collection 3493095) by digestion with *Sal*I and *Xho*I prior to directly cloning the insert into pCAG-floxed-*neo*pA at the unique *Xho*I site. The 2.5 kb insert contained a part of *Wnt5a* 5'UTR, a complete *Wnt5a* ORF and a portion of *Wnt5a* 3'UTR (Figure 1a). Clones carrying the correct plasmid were screened by restriction enzyme digestion and sent for sequencing.

2.2 The transfection of construct into R26CT2S

The transfection was done by using gene juice (Novagen) following the protocol suggested by the manufacturer. Screening for stably-transfected cre-expressing cell line R26CT2S with pCAG-floxed-*neo*pA-*Wnt5a* began with the selection for neomycin resistant clones in 400 µg/ml geneticin (G418, Sigma) followed by exposure to 500 nM 4'-OHT (Sigma) prior to second antibiotic selection for puromycin resistant clones in 1 µg/ml puromycin (Sigma). The clones that survived puromycin were selected as the positive clones for carrying the complete cassette, and were subjected to the downstream application.

2.3 Inducibility and stability of transgenic construct

Inducibility of the *Wnt5a* construct was analysed by Wnt5a overexpression from undifferentiated pCAG-floxed-*neo*pA-*Wnt5a* cell line upon exposure to 400 nM 4'-OHT. Treated and untreated pCAG-floxed-neopA-empty vector cell lines were used as controls. Total RNA was extracted from both the un- and treated lines and was subjected to RT-qPCR for quantitative *Wnt5a* expression.

3.0 Results

A 10.2 kb pCAG-floxed-neopA-Wnt5a plasmid carrying a 2.5 kb of Wnt5a insert (Figure 1a) was produced. The morphology of the cells remained the same upon transfection, the picked colony (Figure 1b) and the transgenic line (c), with high nucleus-cytoplasmic ratio. Upon exposure to 4'-OHT the expression of Wnt5a increased 5-fold as compared to the controls (Figure 1c) indicating the stability and inducibility of the Wnt5a construct.

4.0 Discussion and Conclusion

Inducibility of the transgene expression demonstrates the ability of the Cre/*loxP* system in temporally controlling the expression of Wnt5a at specific time points during neural differentiation process. The conditional transgenic Wnt5a embryonic stem cell line was successfully established, which would be useful and efficient in understanding the roles played by Wnt5a, specifically to monitor the stage dependent activity of Wnt5a during dopaminergic differentiation process.

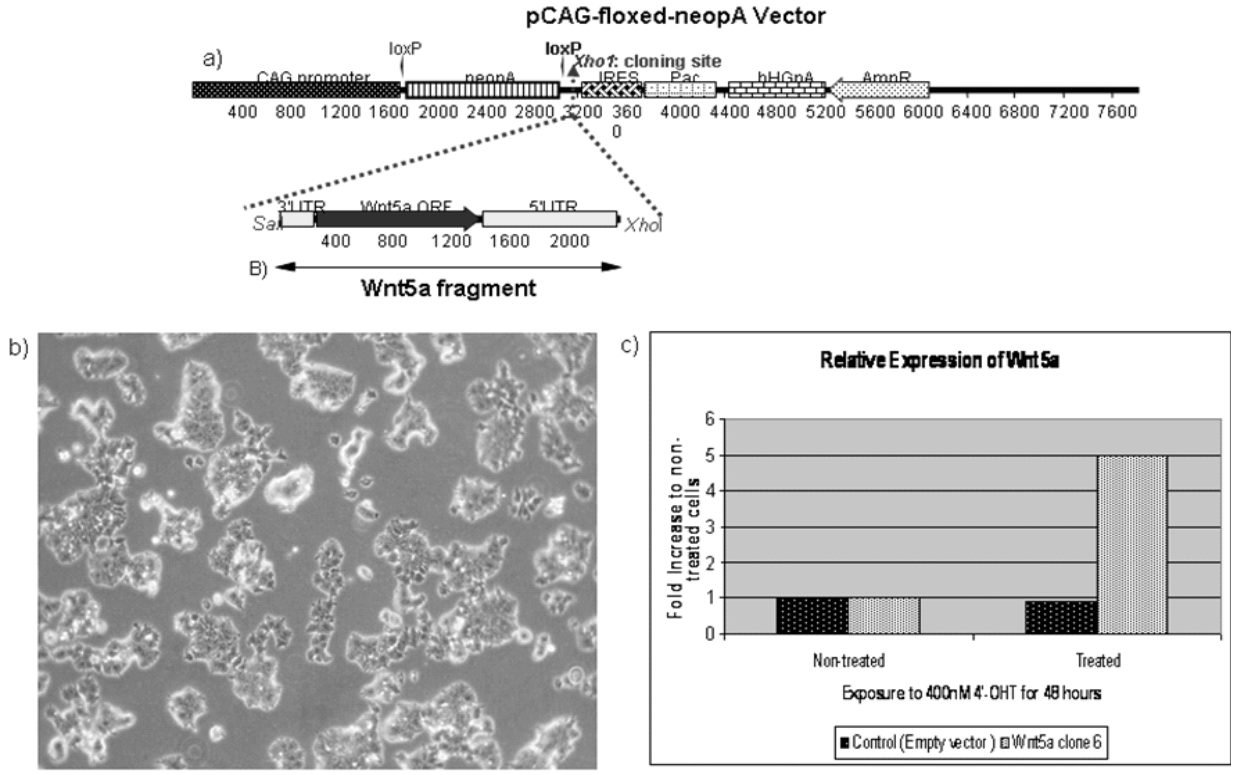

Fig. 1 Establishment of inducible *Wnt5a* cell line. a) Inducible *Wnt5a* construct. b) Morphology of mouse ES cell line carrying *Wnt5a* construct. c) The relative expression of Wnt5a transgene upon activation of Cre by 400 nM 4'-OHT.

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