

Human Pluripotent Stem Cells Serve as an Effective In Vitro Model for Studies of Early Stages of Retinogenesis

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Specification of the neural retina is one of the first events in human development and thus, efforts to study the initial stages of retinal specification have been largely limited. This is particularly true for the earliest event in retinogenesis, the establishment of a definitive retinal fate from a more primitive neural progenitor source. With the advent of human pluripotent stem cells (hPSCs), the complex interplay of transcription factors involved in early events of retinal development from an unspecified pluripotent population can be studied in an in vitro model. To examine this potential, hPSCs were directed to differentiate to a neuroretinal lineage in which a subpopulation of cells adopts a definitive retinal fate whereas others develop toward a forebrain lineage. Samples were collected over the first month of differentiation, starting from the undifferentiated state through when cells acquired either retinal or non-retinal forebrain identities and gene expression patterns were characterized using immunocytochemistry and quantitative RT-PCR. Results demonstrated that while neural transcription factors such as PAX6, OTX2, and LHX2 were expressed early in development, definite retinal transcription factors such as CHX10 were expressed later in differentiation. Furthermore, the expression of CHX10 was found to be uniquely associated with retinal populations and remained absent from the other neural populations, thereby illustrating the ability of this protocol to uniquely identify and isolate retinal populations and further study them in vitro. Overall, these studies will serve to further understand the specification of a retinal fate from a pluripotent population. Such information will assist in the establishment of more efficient methods to generate retinal cells from hiPSCs for translational purposes, as well as establish hiPSCs as a unique *in vitro* model system for studies of the earliest stages of human development.

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