00402 Novel Retina Specific Laminin Isoforms Recapitulate Retinal Interphotoreceptor Matrix to Generate Human Embryonic Stem Cell-derived Photoreceptors

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Aims: Photoreceptors could play a crucial role in retinal cell therapy to treat vision loss. The use of retinal organoid has been hailed to be the established in vitro retinal differentiation method to generate photoreceptors from the pluripotent stem cells. However, this approach is evidently limited by its reproducibility and efficiency level during retinal organoidogenesis. Furthermore, the existing retinal organoid based protocols are not chemically defined and xenogen-free, which include the use of nonhuman animal serum and Matrigel®, a murine Engelbreth-Holm-Swarm sarcoma extract that are not considered clinically safe. Therefore, our aim is to generate clinically safe functional photoreceptors that could potentially restore patient's vision.

Methodology: Currently, we have developed a retinal organoid free method to robustly generate photoreceptor progenitors from human embryonic stem cells (hESCs). Our novel alternative approach employs the use of the human recombinant retina-specific laminin isoforms to recapitulate the retinal interphotoreceptor matrix environment. With the support of an analogous retina matrix like surface, the hESCs are being efficiently differentiated towards photoreceptor progenitor cells. Unlike retinal organoid, this chemically defined and xenogen-free method does not involve replating and manual dissections.

Result: This laminin based differentiation method consistently generates photoreceptor progenitors in every batch of differentiation. These hESCs-derived photoreceptors are shown to be positive for PAX6, VSX2, CRX and RCVRN as early as Day 30, based on transcriptome and immunocytochemical analyses. In contrast, the pluripotency and teratoma transcript markers are shown to be drastically downregulated, suggesting reduced associated risk of teratoma formation.

Conclusion: We provide an alternative differentiation method that does not require the formation of retinal organoid. This xenogen-free and chemically defined protocol is compatible with Good Manufacturing Practice condition and reproducibly promotes hESCs to photoreceptor progenitor lineage. Hence, these results suggest that our method may constitute an important step towards the future use of hESC-derived photoreceptors to treat vision loss.