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Analysis of YFP-Laminin Engineered Retinal Progenitor Cell Migration

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♦ Abstract

Retinal progenitor cell (RPC) transplantation remains a promising reparative paradigm for restoration of damaged retinal tissue. To initiate migration, cells disassemble their hemidesmosome remodel their cytoskeleton and crawl in contact with extra cellular matrix. We hypothesized that RPCs could be engineered to express flourescent laminin and that deposition of laminin-332 (LM-332) is a marker of RPC motility behavior. Here we are using a laminin and yellow fluorescent protein fusion to visualize the migration of RPCs in-vitro. This could be applied in vivo to track cells transplanted in the retina or during organogenesis to visualize migration and ECM modeling. Furture studies may investigate the dynamics of these engineered Laminin-YFP RPCs.

♦ Introduction

There are numerous processes that influence the migration of cells; one of which is chemotaxis. Chemotaxis is the process whereby cells move in a particular direction as a result of the chemicals in their environment. It is either positive or negative depending on direction of migration with respect to the chemical in question; it is positive if the cell is moving in the direction of the chemical and negative if its movement is away from the chemical (1).

Cells communicate this information through their cell surface protein receptors called integrins. The integrins form a major part of a family of cell adhesion molecules (CAMs), which enable cells to bind to their extracellular matrix and link to their cytoskeletons or other cells (2).

For cells to migrate, they must disassemble their hemidesmosome (stable adhesive matrix structure), change their cell-to-cell and cell-to-matrix interactions, and crawl from the margin on a provisional extracellular matrix, which includes laminin (2).

Retinal progenitor cells (RPCs) isolated from post-natal day 1-5 mice can be isolated and expanded in culture. RPCs are the primary cell type of retinal development and are easy to isolate and study . RPCs also migrate spontaneously in culture and also when transplanted into retinal tissue (3).

LM-332 stabilizes cell adhesion and is expressed by a range of migrating cells. We predicted that migrating RPCs would deposit laminin during migration. The functions exhibited by LM-332 include cell adhesion and motility and have been investigated by a number of laboratories. Results to date indicate that proteolytic processing of LM-332 has a profound impact on its functions (3).

During retinal degeneration, photoreceptor cells die leading to functional loss and loss of vision (4). A potential therapy is to transplant retinal progenitor cells into the retina to replace damaged photoreceptor cells. Materials developed in this study will allow us to engineer cells that can be tracked during migration in vitro and in vivo by imaging YFP-laminin deposition. This work will help improve understanding of the migratory mechanisms involved in stem cell transplantation therapies.

♦ Materials & Methods

mRPC Cell Culture

Retinas were isolated from post-natal days 0–3 enhanced green fluorescent protein positive (GFP^+) transgenic mice (C57BL/6 background). Pooled retinas were dissociated by mincing, and digested with 0.1% type 1 collagenase (Sigma–Aldrich; St. Louis, MO) for 20 min. The liberated mRPCs were passed through a 100 µm mesh filter, centrifuged at 850 rpm for 3 min, re-suspended in culture medium Neurobasal (NB; Invitrogen-Gibco, Rockville, MD) containing 2 mm l-glutamine, 100 mg/ml penicillin–streptomycin, 20 ng/ml epidermal growth factor (EGF; Promega, Madison, WI) and neural supplement (B27; Invitrogen-Gibco) and plated into culture wells (Multiwell, Becton Dickinson Labware, Franklin Lakes, NJ). Cells were provided 2 ml of fresh culture medium on alternating days for 3 weeks until mRPCs were visible as expanding non-adherent spheres. mRPCs were passaged 1:3 every 7 days. (redenti et al 2009)

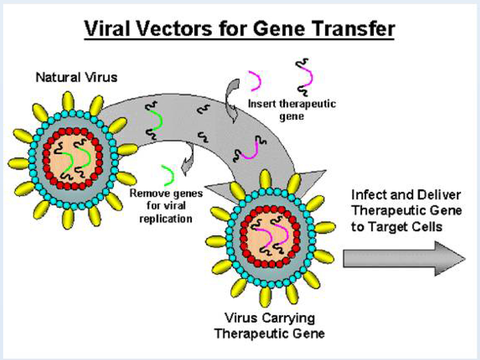
mRPC Transfection
The YFP-laminin adenovirus was borrowed from a the lab of Dr. Jones (5).

To generate virus encoding YFP-tagged full-length Laminin 332, laminin cDNA was cloned into the pYFP-N vector. This produces a fusion protein consisting of the laminin and YFP. This cassette was subcloned into the pENTR-4 vector (Invitrogen) (5).

Mammalian cell Transduction procedure
1.Plate RPCs in media
2.On the day of transduction thaw adenoviral stock and dilute if
3.necessary in complete media
4.Remove the culture media from cells. Swirl the plate to disperse the medium incubate overnight.
5.Following day remove media containing virus and replace with fresh medium.

FBS will be used to stimulate migration in petri dish culture and migration will also be studied in retinal slice in vitro.

Fig 2: mRPC Transfection



http://www.genecure.com/technology.html

♦ Results

Fig 1: mRPC Cell Culture

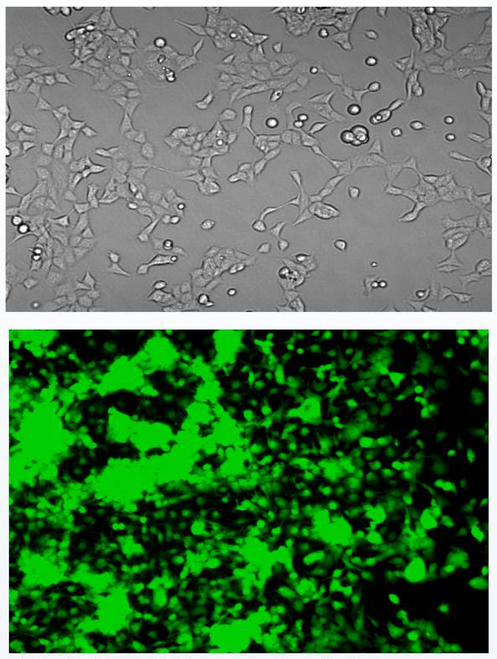
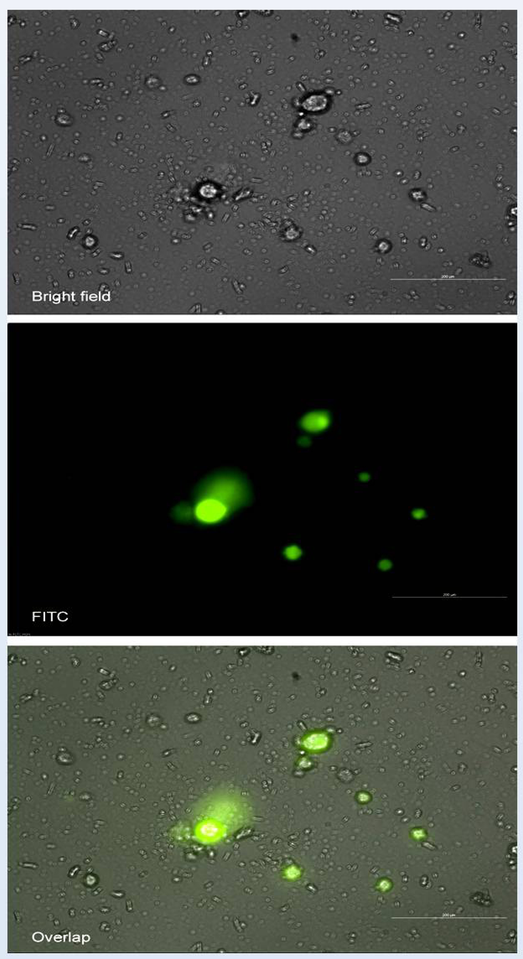


Fig 1: 10 X GFP expressing Retinal Progenitor cells (RPCs)

Fig 3: mRPC YFP-Laminin Trail with Migration



Top) Birght-field 20x image of YFP-Laminin transfected RPC. (midfdle) FITC (GFP) image showing GFP cell soma and surrounding YFP-Laminin deposits. (bottom) overlay of above. Scale 100 microns.

♦ Conclusions

1.mRPCs can be isolated and expanded in culture.

2. mRPCs can be transfected with a YFP-laminin vector.

3. mRPCs containing the YFP-laminin vector secret a YFP-laminin trail during migration in vitro.

4. Using florescence microscopy paths of mRPC migration in vitro can be visualized over time.

♦ Future Directions

1. YFP-laminin transfected RPCs can be stimulated to migrate with specific chemokines and trajectories mapped using fluorescence microscopy.
2. Intensity of YFP laminin can be analyzed to determine level of deposition in response to different migratory cues.
3. Migration YFP-laminin expressing RPCs can be mapped during migration when transplanted into retina to reveal migratory pathways.

♦ References

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