MAP Cell Binding Domain to Attach R28 Retinal Stem Cells to RCS Eyecups Over Time

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INTRODUCTION

Several previous structural and cellular attachment analyses were performed on mussel adhesive protein (MAP) and derivatives. MAP is marketed as a cellular attachment agent for a variety of cell types to various surfaces (1 and references therein). The MAP-repeating decamer (MAP-RD; A-K-P-S-Y-HYP-HYP-T-DOPA-K) and other MAP-derived peptides suggest that the L-DOPA residues are responsible for surface attachment ability. It has also been shown that the lysine-alanine-lysine (K-A-K) residues permit the cellular attachment to MAP films. Structural analysis of several peptides derived from MAP indicated that K-A-K amino acid segments were structurally conserved (2). Cyclic peptides were then designed that contained L-DOPA for the surface adhesion process and the K-A-K regions cellular attachment (DOPA-G-C-G-G-K-A-K-G-C [cycDOPA]). It was shown that cycDOPA formed thin uniform films capable of specific cellular attachment of MOLT-4 cells (2).

The R28 cell is an E1A immortalized retinal precursor cell. When injected into the eyes of dystrophic Royal College of Surgeons (RCS) rats, R28 cells have been shown to survive and migrate after an initial settling within different regions of the retina (3,4). In addition, since R28 cells offer the potential to differentiate and replace damaged neurons, they are also being examined to better understand the pathology of retinal degenerative diseases (5). Since these stem cells offer potential retinal and nerve cell replacement or regeneration, the ability to attach them could provide a mechanism to further these efforts. Prior study examined the ability of cycDOPA films to attach R28 cells to tissue culture plastic. Comparing surface areas covered with R28 cells, assessments regarding attachment abilities were made. R28 cells attached in the greatest number to the K-A-K containing peptide, cycDOPA. When compared to the control surfaces, R28 cells covered an average of 4.5 times the surface area. In addition, physical examination of the surfaces show that multiple layers of cells were found in some areas within the cell wells containing cycDOPA, with some indicating differentiation of the cell (6). For this study, MAP peptides were used to measure their ability to attach R28 cells to denuded inner globes harvested from RCS rats.

MATERIALS AND METHODS

Peptides were synthesized and purified using HPLC and mass spectrometry (SynPep, Dublin, CA) including a cyclic peptide (DOPA-G-G-C-G-K-A-K-G-C; cycDOPA) and a linear peptide (DOPA-K-A-K-P; KAK). MAP was obtained as CELL-TAK™ from BD Biosciences (Bedford, MA).

Six male two-month-old Royal College of Surgeons rats were sacrificed, globes removed, anterior eyecups excised, retinae detached, and posterior eyecups were then placed in a 12 cell well plate in 1.0 ml PBS. DOPA containing peptides, DOPA-G-G-C-G-K-A-K-G-C; cycDOPA; DOPA-K-A-K-P, KAK; and CELL-TAK, were obtained and prepared at a concentration of 1.0 mg/ml in 5% acetic acid, PBS was aspirated from the wells, 38.0 µl of peptide added to 962 µl of 0.1 M sodium bicarbonate solution (pH=8.29); yielding 2.0 µg/cm^2. The resulting peptide/sodium bicarbonate mixtures were placed on the eyecup in each cell well, so that there were three eyecups with each treatment, and incubated at 4^{o}C for an hour. The peptide/sodium bicarbonate solution was aspirated from the wells and R28 cells, labeled with green fluorescent protein (GFP) were applied in 1.5 ml DMEM (LifeTechnologies, Grand Island, NY) to each eyecup at a concentration of 6.67x 10 5 cell/ml. The well plate was incubated in a 5% CO_2 95% air incubator at 37^{o}C and rows of treatments were fixed with 3% gluteraldehyde in PBS at 24, 48, and 72 hours. Eyecups were examined with a Leica fluorescence microscope and GFP + cells were counted in 8 high power fields (HPF) adjacent to the optic disk and totaled for each treatment.

RESULTS AND DISCUSSION

Comparing surface areas covered with R28 cells, assessments regarding attachment abilities were made. MAP attached the lowest number of cells, rinsing of the surfaces removed any loosely associated cells. This phenomenon has been observed when using this particular film preparation (1 and references therein). R28 cells attached in the greatest number to the KAK containing agents, especially the linear KAK peptide throughout the 24, 48 and 72 hour times.

HPF counts of the samples were taken at 24 hour intervals. KAK proved to be not only the best of the adhesives used but it also gave consistent results after 24, 48, and 72 hours, while the other adhesives appeared to lose ability to keep cells attached.

Physical examination of the eyecup surfaces show that multiple layers of cells were found in some areas, with some indicating possible differentiation of the cell. Since it has been shown that these peptides enhances R28 cellular attachment, and possible controlled differentiation, continuing studies are utilizing antibody labelling techniques to identify cellular differentiation.

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