Supplemental figure 1. PVR primary cultures. (A). Passage 0 C-PVR cells from PVR-01 in culture (Scale bar 500 µm). (B) and (C) show the same culture using a higher magnification. Scale bar 100 µm. (D) The same C-PVR culture at passage 3. (E) and (F) show the culture using higher magnification.
Supplemental figure 2. Establishment of culture conditions to support C-PVR growth. C-PVR cells from PVR-05 cultured with (A) Basal medium (EBM2) were not conducive to survival. (B) 12% FBS alone resulted in cell growth. Treatment with Heparin (C), IGF (D), FGF (E), Ascorbic Acid (F), Hydrocortisone (G), EGF (H), VEGF (I) or PDGF-B (L) were not enough to support C-PVR growth. (J) C-PVR medium (12% FBS, Heparin, IGF, FGF, Ascorbic Acid, Hydrocortisone, EGF and VEGF) permitted robust growth. (K) Treatment with all the growth factors listed for (J), except 12% FBS, were not sufficient to support C-PVR growth. Treatment with TGF-β1 (M), TGF-β2 (N), TGF-β3 (O) or the combination of all of them together (P) induced a change in cell morphology (elongated and flat cells) but not growth. (Q) 10 µM and (R) 100 µM Compound E, a gamma-secretase inhibitor did not produce confluent cultures. (S) Graphs shows quantification of cell number. Error bars represent standard error of the mean. Scale bar 100 µm.
Supplemental figure 3. Ki67 staining of PVR membranes and their corresponding C-PVR cultures. Membranes from PVR-03 and PVR-05 (A and B, respectively) and corresponding C-PVR cultures (D and E, respectively) were processed for immunohistochemistry using an antibody against Ki67 (in red) to detect cell proliferation and DAPI for label nuclei (in blue). There was a non-significant trend for higher proliferation in PVR-05 membrane and its corresponding C-PVR culture compared to PVR-03. C and F show the quantification of the assay. Error bars represent standard error of the mean. Scale bar 100 µm.
Supplemental figure 4. Effect of MTX on cell density. Phase contrast and Hoechst-stained immunofluorescence images of C-PVR cells from PVR-05 after six weeks of culture without MTX (A) and with 100 µM (C), 200 µM (E), and 400 µM (G) MTX. (I) Quantification of cell density showing a significant reduction in cell density after treatment with MTX. Error bars represent standard error of the mean. Scale bar 100 µm.
Supplemental figure 5. Proliferative activity assessment by Ki67 of C-PVR and ARPE19 cultures treated with methotrexate (MTX), dexamethasone (DEX), and daunorubicin (DNR). C-PVR from PVR-05 (A) and ARPE19 (B) were treated with different MTX concentrations (100 µM, 200 µM, and 400 µM) and control with no MTX. C-PVR were treated with different concentrations of DEX (0.02 mg/ml, 0.2 mg/ml, and 2.0 mg/ml) and control with no DEX (C). C-PVR were treated with different DNR concentrations (1.5 nM, 15 nM, and 150 nM) and the control with no DNR (D). Error bars represent standard error of the mean.
Supplemental figure 6. Scratch-wound assay of C-PVR treated with MTX. C-PVR cells from PVR-05 were cultured using different MTX concentrations (100 µM, 200 µM, and 400 µM) and control with no MTX. Images were taken 0 hours (A-D), 24 hours (E-H), and 48 hours (I-L) after wounding. Significant cell migration was detected after 24 and 48 hours, but no difference in cell migration between MTX treatment and controls were detected. (M) shows the quantification of the assay. Error bars represent standard error of the mean. Scale bar 100 µm.
Supplemental figure 7. Effect of TNF-α on C-PVR migration. Migration for C-PVR from PVR-05 was detected by scratch assay. Images were taken 0 hours (A-G), 24 hours (H-N), and 48 hours (O-U) after specific treatments. C-PVR were treated with control (A), vehicle (B), 400 µM MTX (C), 1 ng/ml TNF-α (D) and 10 ng/ml TNF-α (F), 1 ng/ml TNF-α and 400 µM MTX (E) and 10 ng/ml TNF-α and 400 µM MTX (G). (V) Graph shows quantification of wound closure. Error bars represent standard error of the mean. Scale bar 100 µm.
Supplemental figure 8. Assessment of caspase activation of C-PVR treated with MTX after 2 weeks of treatment. Hoechst-stained immunofluorescence images of C-PVR cells from PVR-05 after two weeks of culture without MTX (A, B, C) or with 100 µM (D, E, F), 200 µM (G, H, I), and 400 µM (J, K, L) MTX. Scale bar 100 µm.
Supplemental figure 9. Assessment of caspase activation of C-PVR treated with MTX after 4 weeks of treatment. Hoechst-stained immunofluorescence images of C-PVR from PVR-05 after four weeks of culture without MTX (A, B, C) and with 100 µM (D, E, F), 200 µM (G, H, I), and 400 µM (J, K, and L) MTX. Scale bar 100 µm.