Retinoid Uptake, Processing, and Secretion in Human iPSRPE Support the Visual Cycle

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FIGURE 2. Brightfield images of cultured iPS cells and iPS-RPE. (A) iPS cells prior to differentiation. The cells in the colonies maintain typical round pluripotent stem-cell morphology. (B) After passage, iPS-RPE cells revert to fibroblastic morphology, losing their classic hexagonal RPE morphology and pigmentation. (C) iPS-RPE passage 6 regained hexagonal morphology and pigment within 4 weeks after passage. (D) Highly pigmented iPS-RPE passage 6 after 6 months in culture. Magnification ×200.
FIGURE 3. Expression of RPE genes in iPS-RPE. Transcripts for LRAT, RPE65, CRALBP, and PEDF were analyzed by RT-PCR. Gene expression was not detected in iPS cells cultured in nondifferentiation conditions (A), while iPS-RPE (B) showed expression of all analyzed RPE genes after 4 weeks in culture. GAPDH was included as a control. Visual cycle proteins CRALBP (D, F) and RPE65 (H, J) were detected by immunocytochemistry in iPS-RPE (F, J) after 5 weeks in culture, but not iPS cells cultured in nondifferentiation conditions (D, H). DAPI labeling of iPS cells is shown in C and G. DAPI labeling of iPS-RPE is shown in E and I. Western blot detection of CRALBP, RPE65, and LRAT (indicated by arrow) further confirms protein expression at the expected molecular weights (35 kD, 65 kD, and 25 kD, respectively) in L, M, and N.
FIGURE 4. Retinyl ester synthesis in iPS-RPE cultures. iPS and iPS-RPE cells cultured for 4 weeks were incubated with 10 µM all-trans retinol for 24 hours. Retinoids were extracted from the cultured cells for analysis by gradient HPLC. (A) Chromatogram of authentic retinyl ester standards. Peak 1, 11-cis retinyl palmitate; Peak 2, all-trans retinyl palmitate. (B) Chromatogram of retinoids extracted from iPS cell cultures incubated without 10 µM all-trans retinol. (C) Chromatogram of retinoids extracted from iPS-RPE cultures incubated with 10 µM all-trans retinol. On the right are the absorbance spectra corresponding to the retinyl ester standard and experimental peaks; the peaks corresponding to all-trans retinyl palmitate are indicated by 2, 3, and 4, respectively. All retinoids were identified by comparison with retention time and absorbance spectra of authentic retinoid standards and quantified by retinoid standard curves. (D) Chart representing all-trans retinyl palmitate extracted from iPS-RPE cells treated for 24 hours with 10 µM all-trans retinol delivered either with 2% BSA or with 2% BSA plus 15% FBS. Retinoids were extracted and analyzed by HPLC. The iPS-RPE in the treatment groups with BSA and BSA + FBS synthesized all-trans retinyl palmitate in the amounts of 1724 ± 673 pmol/mg protein and 2942 ± 551 pmol/mg protein, respectively. Only trace amounts of all-trans retinyl palmitate (20 ± 5 pmol/mg protein) were detectable in control iPS-RPE cells incubated without all-trans retinol. Monitoring λ = 325. Data are expressed as mean ± SEM. *P < 0.05 and †P = 0.06
FIGURE 5. LRAT facilitates synthesis of all-trans retinyl ester synthesis in iPS-RPE. Whole-cell homogenate from iPS-RPE cultured for 4 weeks was incubated for 1 hour with all-trans retinol in the presence or absence of NEM. Retinoids were extracted for gradient HPLC analysis. (A) Chromatogram of authentic retinyl ester standards. Peak 1, 11-cis retinyl palmitate; Peak 2, all-trans retinyl palmitate. (B) Chromatogram of retinyl esters (Peak 3) extracted from 500 µg iPS-RPE homogenate incubated with 10 µM ATOL. (C) Chromatogram of retinyl esters (Peak 4) extracted from 500 µg iPS-RPE homogenate incubated with 10 µM ATOL and NEM. Note the reduction of all-trans retinyl palmitate (Peak 4) in the presence of NEM, a specific LRAT inhibitor. On the right are the absorbance spectra corresponding to the retinyl esters, respectively. (D) Increasing total amounts (100, 250, and 500 µg) of iPS-RPE homogenate protein were incubated for 1 hour with 10 µM all-trans retinol with or without NEM. The bar graph indicates synthesis of all-trans retinyl palmitate increased as the amount of iPS-RPE homogenate protein increased. As shown, chemical inhibition with NEM reduced the synthesis of all-trans retinyl palmitate by 90%. Retinoids were not detected in iPS-RPE homogenate controls incubated without all-trans retinol. All retinoids were identified by comparison with retention time and absorbance spectra of authentic retinoid standards and quantified by retinoid standard curves. Monitoring λ = 325. Data are expressed as mean 6 SEM. *P < 0.05.
FIGURE 6. Cultured iPS-RPE synthesize and release retinaldehydes from ATOL. iPS and iPS-RPE cells cultured for 6 months were incubated with 10 µm all-trans retinol for 24 hours. Retinoids were extracted from the culture media for analysis by gradient HPLC. (A) Chromatogram for authentic retinaldehyde standards. The retinaldehydes standards are identified as follows: Peak 1, 13-cis retinaldehyde; Peak 2, 11-cis retinaldehyde; Peak 3, 9-cis retinaldehyde; Peak 4, all-trans retinaldehyde. Insets to the right are representative spectra for each retinaldehyde standard. (B) Chromatogram for culture media extract from IPS-RPE cells incubated without all-trans retinol. (C) Chromatogram for culture media extract from iPS cells incubated with all-trans retinol. The peak that appeared just before the peak for 11-cis retinaldehyde did not correspond to any known retinoids, therefore the absorbance spectrum is not included. (D) Chromatogram for culture media extract from iPS-RPE cells incubated with all-trans retinol. Peaks corresponding to the retention time of 11-cis retinaldehyde, 9-cis retinaldehyde, and all-trans retinaldehyde were detected in the iPS-RPE media. Peak 5 has a retention time of 14.9 minutes that correlates with the retention time of 11-cis retinaldehyde in the standard run shown by Peak 2 in A. The absorbance spectrum49–51 for this peak further indicates the presence of 11-cis retinaldehyde in the culture media. Quantification of 11-cis retinaldehyde peak results in 188 ± 88 pmol/mg of iPS-RPE protein. Monitoring λ = 365.
FIGURE 7. iPS-RPE synthesized and released 11-cis RAL from exogenous all-trans retinol. iPS-RPE cultured for 5 months and iPS cells were incubated with all-trans retinol for 24 hours. Retinoids were then extracted from the culture media and analyzed by isocratic HPLC. (A) Chromatogram of authentic retinaldehyde standards. Peak 1, 13-cis RAL; Peak 2, 11-cis RAL; Peak 3, 9-cis RAL; Peak 4, all-trans RAL. (B) Chromatogram of media extract form iPS cells incubated in the presence of all-trans retinol. (C) Chromatogram of media extract from iPS-RPE incubated with all-trans retinol. (D) Chromatogram of authentic 11-cis RAL standard. (E) Chromatogram of retinoid extract from media of iPS-RPE incubated with all-trans retinol combined with authentic 11-cis RAL. The (*) marks peaks corresponding to 11-cis RAL. Insets are the absorbance spectra for the labeled peaks in the chromatograms. Monitoring $\lambda = 365$. (B) does not include an absorbance spectrum because a peak corresponding to 11-cis RAL was not detected in the iPS cell media.
Results/Conclusion

- Cultured iPS-RPE expresses visual cycle genes LRAT, CRALBP, and RPE65. After incubation with all-trans retinol, iPS-RPE synthesized up to 2942 ± 551 pmol/mg protein all-trans retinyl esters. Inhibition of LRAT with N-ethylmaleimide (NEM) prevented retinyl ester synthesis. Significantly, after incubation with all-trans retinol, iPS-RPE released 188 ± 88 pmol/mg protein 11-cis retinaldehyde into the culture media.

- iPS-RPE develops classic RPE characteristics and maintains expression of visual cycle proteins. The results of this study confirm that iPS-RPE possesses the machinery to process retinoids for support of visual pigment regeneration. Inhibition of all-trans retinyl ester accumulation by NEM confirms LRAT is active in iPS-RPE. Finally, the detection of 11-cis retinaldehyde in the culture medium demonstrates the cells’ ability to process retinoids through the visual cycle. This study demonstrates expression of key visual cycle machinery and complete visual cycle activity in iPS-RPE.