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Effect of Retinoic Acid Treatment on the Retinoic Acid Signaling Pathway in a siRNA-Based Aniridia Limbal Epithelial Cell Model, in Vitro

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Abstract : Purpose: Congenital aniridia is characterized by PAX6 haploinsufficiency, and aniridia-associated keratopathy (AAK). In AAK, limbal stem cell deficiency and impaired wound healing are wiPurpose: Congenital aniridia is characterized by PAX6 haploinsufficiency and aniridia-associated keratopathy (AAK). In AAK, limbal stem cell deficiency and impaired wound healing are widely observed in patients, which might be associated with an imbalanced retinoic acid (RA) signaling pathway. In the previous studies, we demonstrated the relationship between PAX6 and the altered expression levels of key markers in the RA signaling pathway to retinol treatment. The present study evaluates the gene and protein expression levels in an in vitro small interfering RNA (siRNA) PAX6 knockdown aniridia limbal epithelial cell model following retinoic acid treatment. This study targets the direct effects of active RA products and their association with key regulators of the RA signaling pathway in siRNA PAX6 knockdown LECs. Methods: Primary human limbal epithelial cells (LECs) were knocked down by siRNA treatment to mimic PAX6 deletion in congenital aniridia (n=8). This was followed by 0µM, 1µM, and 5µM retinoic acid treatment applied in both siRNA PAX6 control and knockdown groups. After 48h incubation, paired box 6 (PAX6), alcohol dehydrogenase 7 (ADH7), aldehyde dehydrogenase 1 family member A1 (ALDH1A1), cytochrome P450 family 26 subfamilies A member 1 (CYP26A1), retinol-binding protein 1 (RBP1), cellular retinoic acid binding protein 2 (CRABP2), fatty acid binding protein 5 (FABP5), retinoid X receptor alpha (RXRA), retinoid X receptor beta (RXRB), retinoic acid receptor alpha (RARA), retinoic acid receptor beta (RARB), peroxisome proliferator-activated receptor gamma (PPARG), vascular endothelial growth factor A (VEGFA) mRNA levels have been determined using qPCR and protein levels by ELISA or western blot. Results: PAX6, ADH7, ALDH1A1, FABP5 mRNA levels and PAX6, ADH7, FABP5, PPARG2 protein levels were significantly lower in the PAX6 knockdown group, than in controls ($p<0.001$, $p=0.018$, $p=0.015$, $p<0.001$; $p<0.001$, $p=0.003$, $p<0.001$, $p=0.007$). PPARG mRNA level was significantly higher in the PAX6 knockdown group than in controls ($p=0.012$). CYP26A1 mRNA expression was upregulated using 1 µM and 5 µM RA treatment in both the PAX6 control ($p<0.001$; $p<0.001$) and the PAX6 knockdown group ($p=0.001$; $p=0.002$). CRABP2 mRNA expression in the PAX6 knockdown group ($p=0.02$) and protein expression in both groups were downregulated using to 5 µM RA concentration ($p=0.003$; $p=0.02$). RARA mRNA expression in the PAX6 knockdown group ($p=0.023$), RARB mRNA expression in both groups ($p=0.006$, $p=0.001$), and RXRA protein expression in controls ($p=0.007$), were downregulated using 5 µM RA concentration. VEGFA mRNA expression in PAX6 controls was upregulated using 5 µM RA ($p=0.041$). FABP5 to CRAP2 ratio was higher in PAX6 controls than in the PAX6 knockdown group ($p<0.001$). Additionally, the FABP5 to CRAP2 ratio was only upregulated in PAX6 controls using 5 µM RA concentration but not in the PAX6 knockdown group ($p<0.001$). Conclusions: These results reveal a less-responsive FABP5 to CRABP2 ratio in PAX6 knockdown LECs following increased RA concentration, as well as altered expression of key regulators in the RA signaling pathway. Further investigations into the regulatory processes are required to elucidate the role of RA signaling in the development of AAK.

Keywords : congenital aniridia, paired box 6 gene, aniridia-associated keratopathy, retinoic acid signaling pathway
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