P53, CDKN1A, STEAP3, CASP10 Gene expressions in RPTEC/TERT1, HRTPT, and HREC24T from the cell line

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Abstract

- The proximal tubules, located in the kidneys, are one of the main sites of injury to kidneys. This is due to large surface area, increased blood filtrate, and high amounts of mitochondria [1]. There are stem/progenitor cells that are involved in the regeneration of the renal tubules but the mechanisms by which the stem cells repair the tubules is not fully understood. To grasp a fuller understanding of how this process works we are looking at the gene expression of the following genes: P53, CDKN1A, STEAP3, and CASP10 using RT-qPCR. This group of genes are mainly involved in the apoptosis, necrosis, and senesces process of the cells of the human proximal tubules. The cells being used for this study are the RPTEC/TERT1, HRTPT and HREC24T cells. HRTPT are considered stem/progenitor cells and HREC24T considered as differentiated cells are the cell populations isolated from the RPTEC/TERT1 [2]. The mRNA levels for these genes showed minimal to no expression for the HRTPT cells while the significant increase in HREC24T cells compared to the RPTEC/TERT1 parental line. This suggests that the HREC24T cells which possess less stem/progenitor characteristics and predicted to be differentiated cell type may have acquired senescence characteristics, therefore increased expression of these relevant markers involved in senescence and cell death process.

Methods

Cell Culture:

- Flow cytometry

Confluent cultures of the immortalized RPTEC/TERT1 cell line were washed two times with PBS and the cells were detached using Accutase (BD Biosciences) and centrifuged at 2000 rpm at 4 °C for 5 min. The cell pellet was washed once and re-suspended in BD Pharmingen™ stain buffer (BSA). Cells were diluted to 1 x 10^6 cells/µL with the stain buffer. For every 100 µl of cell suspension, 10 µl of fluorescein isothiocyanate (FITC) conjugated CD133 and/or phycoerythrin (PE) conjugated CD24 antibody (Miltenyi Biotec Inc.) was added to the tubes. The cells were incubated for 30 min on ice in the dark and then washed two times with stain buffer and re-suspended in 1mL of stain buffer. Cells were sorted using BD FACS Aria (BD Biosciences). The singlet population was gated as FSC vs SSC following which the selected population was graphed as CD133 vs CD24 plot. Four quadrants were drawn to separate double positive vs single positive cell populations. Based on the compensation measurements, the threshold for positive signal was set to ≤ 1 x 10^3 for both markers at the x and y-axis. For each population, 1 x 10^5 cells were sorted directly into a 24-well plate containing warm culture media. The sorted cell population co-expressing CD133 as well as CD24 markers were named HRTPT or CD133+/CD24+ and the cell population expressing CD24 only were named HREC24T or CD133-/CD24+. The cells were allowed to grow to confluency and expanded for further experiments.

- **RNA Isolation and RT-qPCR:**

Expression of P53, CDKN1A, STEAP3, and CASP10 mRNA was assessed by qRT-PCR. The cells were homogenized with TRI reagent and RNA was extracted using 100ul BCP for every 1000 ul of suspension, RNA was then precipitated with 100% isopropanol and washed multiple times with ethanol. Purified RNA was diluted down to 20 ng/50uL and were subjected to cDNA synthesis using cDNA synthesis (reverse-transcription) protocol describe in the notebook in total volume of 20 ul. Real time PCR was performed using the SYBR Green kit (Bio-Rad Laboratories) with 2 ul of cDNA, 1-2 ul of available Primers for each GOI in a total volume of 20 ul. Amplification was monitored by SYBR Green florescence in a CFX96 Touch Real-Time Detection System (Bio-Rad Laboratories). Cycling parameters consisted of denaturing at 95 degrees Celsius for 15 seconds, annealing at 60 degrees Celsius for 30 seconds, and extension at 72 degrees Celsius for 30 seconds. The gene expression levels of all Gene of interest in this project was normalized to the level of Beta Actin expression.[5]

Figure 1

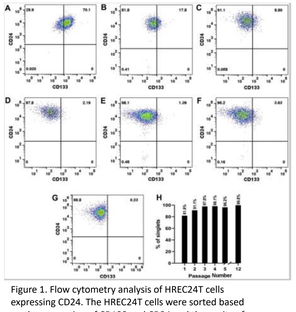

Figure 1. Flow cytometry analysis of HREC24T cells expressing CD24. The HREC24T cells were sorted based on the expression of CD133 and CD24 and the purity of the cells was determined at various passages of cell growth. (A). Analysis of the starting population of CD133$^-$/CD24$^+$ cells in the RPTEC/TERT1 cell line. (B-G). Analysis of CD133$^-$/CD24$^+$ expressing HREC24T cells at passage 1, 2, 3, 4, 5 and 6 respectively. (H). Bar diagram representing the percentage of CD133$^-$/CD24$^+$ expressing cells at various passages of culture. [2]

Figure 2

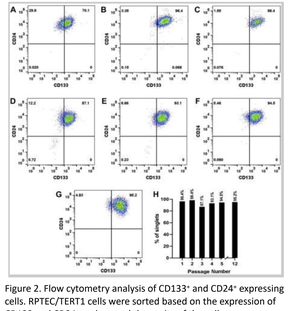

Figure 2. Flow cytometry analysis of CD133$^+$ and CD24$^+$ expressing cells. RPTEC/TERT1 cells were sorted based on the expression of CD133 and CD24 markers and the purity of the cells was determined at various passages of cell growth. (A). Analysis of the starting population of CD133$^+$/CD24$^+$ expressing RPTEC/TERT1 cells. (B-G). Analysis of CD133$^+$/CD24$^+$ co-expressing HRTPT cells at passage 1, 2, 3, 4, 5 and 6 respectively. (H). Bar diagram representing the percentage of CD133$^+$/CD24$^+$ expressing cells within the population of HRTPT cells at various passages of culture. [2]

Introduction

- The renal proximal epithelial cells are of interest in this study because of their location and function within the kidneys. The renal proximal tubules are responsible for the reabsorption of filtrate, active solute secretion, hormone production and the maintenance of homeostasis within the body [3]. Due to the large surface area, increased blood filtrate and high number of mitochondria the proximal tubules are more vulnerable to injury associate with obstruction, hypoxia, aminoglycoside antibiotics, and exposure to carcinogens [1] [2]. Studies have confirmed that stem/progenitor cells play a key role in restoration of the renal tubules post damage [1] [2]. To study how the regeneration process work more closely human kidney derives cell culture models are used [2]. The immortalized human renal epithelial cell line used for this study was one that was recently developed referred to as RPTEC/TERT1. Previously human kidney derived cell cultures did not have all the differentiation features seen in human proximal tubules (HPT) cells but the RPTEC/TERT1 line does possess most of the differentiated characteristics of HPT cells furthermore the RPTEC/TERT1 line showed strong similarities to primary mortal HPT cells, when looking at differential gene expression [2] making it ideal for this study as the expression of the genes P52, CDKN1A, STEAP3, CASP10 were analyzed in the two cell populations found in RPTEC/TERT1 cell line. Studies from this same lab confirmed the presence of both these cells populations in the RPTEC/TERT1 line [2]. The first cell population is HRTPT, which co-expresses cell surface markers CD133 and CD 24 making it the stem/progenitor cell population [2]. The second being HREC24T, which expresses only CD24, meaning these cells were more differentiated and did not contain the stem/progenitor properties seen in CD133+/CD24+[2]. The gene set analyzed was P53, CDKN1A, STEAP3, CASP10. The genes are involved in the following pathways: cell apoptosis, necrosis, and senescence. The P53 gene is tumor suppressor gene that produces the protein p53 inside the nucleus of the cells and plays a large role in controlling cell division and cell death (apoptosis) [5]. CASP10 is a protein coding gene that encodes a protein which is a member of the cysteine-aspartic acid protease family aka as caspase. Sequential activation of caspase plays a central role in the execution-phase of cell apoptosis. CASP10 is also a part of the signaling pathways of inflammation [6]. The gene STEAP3 is also a protein coding gene that encodes a multipass membrane protein that functions as an iron transporter. The encoded protein can reduce both iron (Fe3+) and copper (Cu2+) cations. This protein may also mediate downstream responses to p53, including promoting apoptosis. Deficiency in this gene can cause anemia [6]. CDKN1A is a protein coding gene and encodes a potent cyclin-dependent kinase inhibitor that is responsible for the regulation of the cell cycle progression at G1. The expression of CDKN1A is tightly controlled by the tumor suppressor protein p53 [5]. The goal of this study is to examine the basal mRNA expression levels of P53, CDKN1A, STEAP3, and CASP10 in RPTEC/TERT1, HRTPT and HREC24T cell line to validate array data.

Figure 3

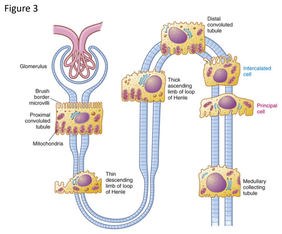

Figure 3. Epithelial Cells of the Various Segments of Nephron Tubules. The brush border and high number of mitochondria in the cells of the proximal convoluted tubule permit reabsorption of 60% of the glomerular filtrate. *Intercalated cells (blue text)* secrete either H^+ (reabsorb $^{HCO-3}$) or reabsorb K^+. *Principal cells (magenta text)* reabsorb Na^+ and water and secrete K^+ [8]

Results

Figure 4

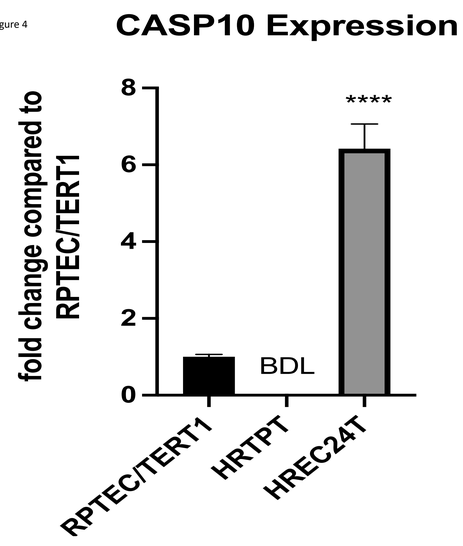

Figure 4. CASP10 expression in CD133+/CD24+, CD133-/CD24+ cell population and in RPTEC/TERT1 cell line. ****p-value </= 0.0001 , BDL denotes "below detection level "

Figure 5

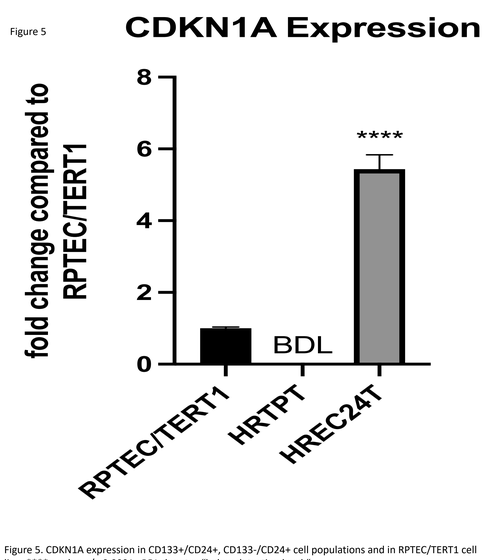

Figure 5. CDKN1A expression in CD133+/CD24+, CD133-/CD24+ cell populations and in RPTEC/TERT1 cell line. ****p-value </= 0.0001 , BDL denotes "below detection level "

Figure 6

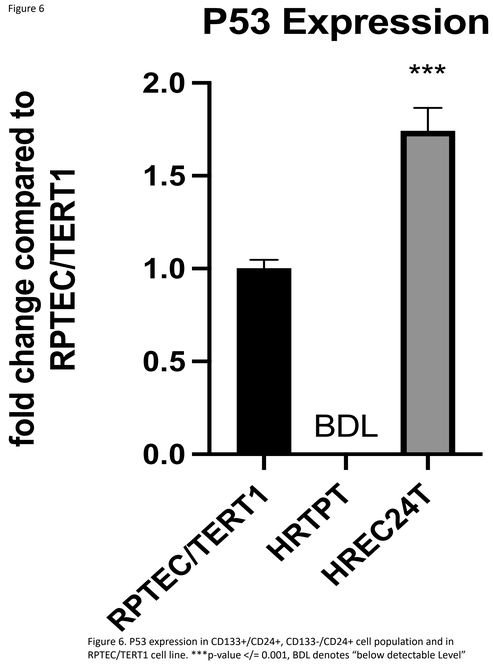

Figure 6. P53 expression in CD133+/CD24+, CD133-/CD24+ cell population and in RPTEC/TERT1 cell line. ***p-value </= 0.001, BDL denotes "below detectable Level"

Figure 7

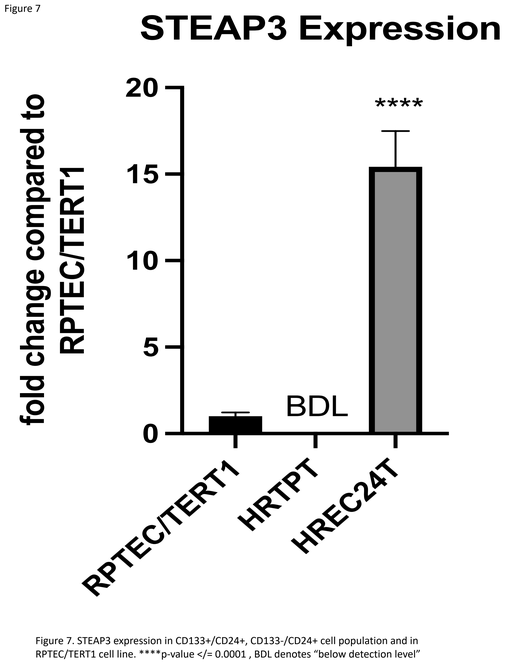

Figure 7. STEAP3 expression in CD133+/CD24+, CD133-/CD24+ cell population and in RPTEC/TERT1 cell line. ****p-value </= 0.0001 , BDL denotes "below detection level"

Conclusions & Discussion

The results of the RT-qPCR displayed gene expression levels of p53 to have a significant increase in expression in the HREC24T cell population while there was a non-detectable level of expression in the HRTPT cell population. CDKN1A also had a significant increase in expression in the HREC24T cell population and with non-detectable expression in the HRTPT. Similarly, STEAP3 and CASP10 both showed significant increase in their expression in the HREC24T cells with non-detectable levels of expression in the HRTPT.

Overall, there was a significant up-regulation in the HREC24T cells and a non-detectable expression levels of these genes in the HRTPT cells. The qPCR results follow the expression patterns displayed by the microarray data analysis performed by this laboratory [7]. Study on the expression on these genes gives important knowledge about the characteristics and cell types of the HRTPT and HREC24T cells. The results obtained from this study may suggest that the HREC24T behave more like differentiated cell type and might possess lower growth and cell division capability than the HRTPT cells.

References

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