PARATHYROID HORMONE TYPE-1 RECEPTOR GENE EXPRESSION ANALYSIS DURING *IN VITRO* MYOGENESIS OF HUMAN SKELETAL MUSCLE SATELLITE CELLS

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BACKGROUND AND AIM

The skeletal muscle function is severely impaired in hypoparathyroidism. The direct effect of parathyroid hormone deficiency in skeletal muscle regeneration has not been fully elucidated. Satellite cells are the stem cells of the skeletal muscle, responsible for skeletal muscle regeneration. The aim of this work is to analyze the expression of parathyroid hormone type-1 receptor (PTH1R) during *in vitro* myogenesis of human satellite cells (hSCs).

MATERIAL & METHODS

The hSCs were isolated from healthy human skeletal muscle biopsies and characterized by analyzing the presence of gene and protein of the main nuclear transcription factor PAX7, by qualitative PCR and flow cytometry, respectively. To establish a model of *in vitro* myogenesis, hSCs were grown in differentiation medium and we have characterized the myogenic phenotype, by verifying the presence of multinucleated cells using phase contrast microscopy microscopy and the expression of terminal differentiation marker, Myosin Heavy Chain (MHC) protein using laser scanning g confocal microscopy (LSCM). The real time TaqMan qPCR was performed to analyse the expression of PTH1R gene in human skeletal muscle tissues (hSMTs) and their derived SCs using human specific probe and primers. The amplicon obtained with the PTH1R probe and primers used in above assay, has been verified for specificity by sequencing. To detect variation in expression of MHC and PTH1R gene during *in vitro* myogenesis, the hSCs were grown for T0-3-6-9 days in myogenic differentiation medium and real time TaqMan qPCR was performed.

RESULTS

Primary culture and characterization of hSCs

- ✓Satellite cells were isolated from 3 human skeletal muscle biopsies and primary cell lines were established
- ✓The isolated hSCs express marker PAX-7 gene and protein, demonstrated by flow cytometry and qualitative PCR, respectively

Biopsies	Skeletal muscle Name	Age	Sex	Derived Satellite Cells
Human Skeletal Muscle Tissue 1 **(hSMT1)**	Abdominus rectus muscle 	50	Female	**hSC1**
Human Skeletal Muscle Tissue 2 **(hSMT2)**	Quadricipite muscle 	57	Male	**hSC2**
Human Skeletal Muscle Tissue 3 **(hSMT3)**	Soleo muscle 	52	Male	**hSC3**

Table 1. Information of human skeletal muscle biopsies and their derived satellite cells taken for analysis

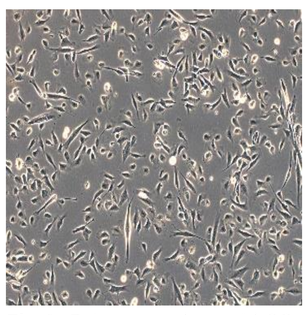


Fig 1. Primary cultured hSCs observed in Phase contrast microscopy, objective 10x

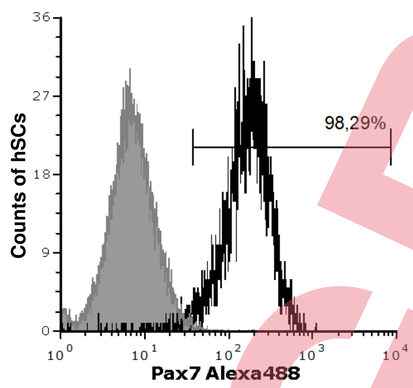


Fig2. Phenotypic characterization of hSCs by analyzing the presence of marker protein PAX7 using flow cytometry

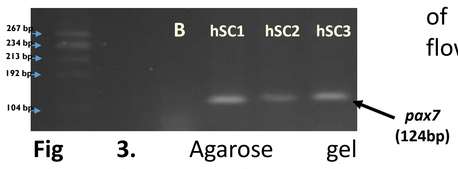


Fig 3. Agarose gel electrophoresis confirming the presence of marker pax7 gene in cultured hSCs

Myogenic differentiation of hSCs

- ✓The myogenic phenotype, the multinucleated cells & expression of MHC protein and gene were observed after myogenic differentiation induction
- ✓The obtained results shows the suitability of the myogenic differentiation cellular model for further analysis

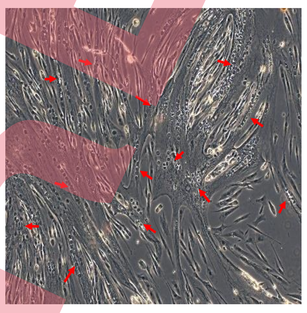


Fig 4. Multinucleated cells (red arrows) observed in phase contrast microscopy, objective 10x, after 7 days of myogenic induction

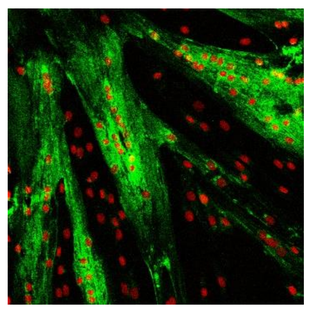


Fig 5. Representative image in LSCM of the MHC protein (green), nuclei (red/orange), objective 20x after 7 days of myogenic induction

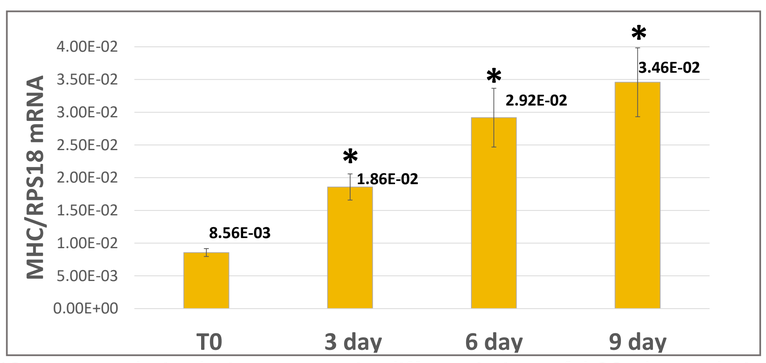


Fig 6. MHC gene analysis during myogenic differentiation of hSCs by real time quantitative TaqMan qPCR, normalized with housekeeping gene RPS18 (Average ± SD, Significance VS. T0)

* p<0.001

PTH1R gene expression in hSMTs and hSCs

- ✓The human skeletal muscle tissue and their derived satellite cells express PTH1R gene

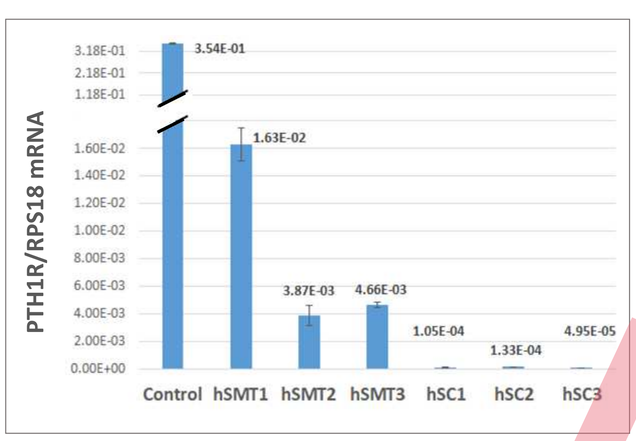
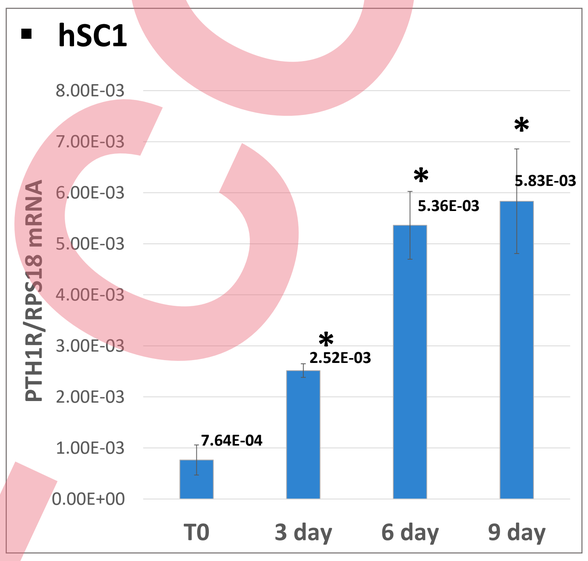


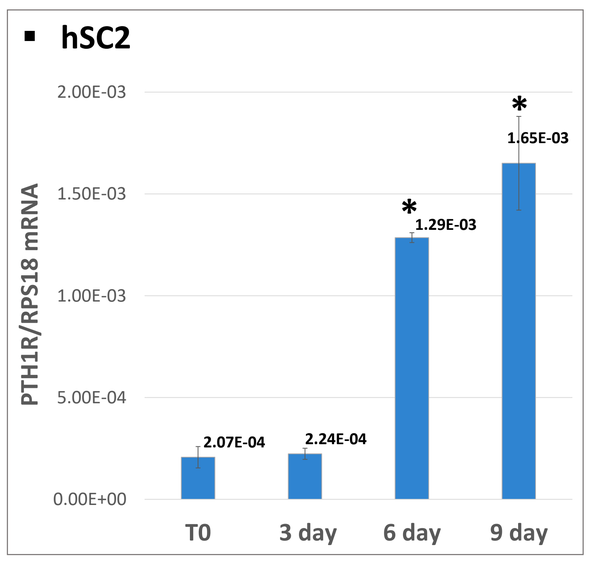
Fig 7. PTH1R gene analysis in hSMTs and hSCs, and positive control human kidney cDNA by real time TaqMan qPCR, normalized with housekeeping gene RPS18

PTH1R gene expression analysis during myogenic differentiation of hSCs

- ✓ The results of PTH1R gene expression analysis have shown a significant increase of this receptor during myogenic differentiation vs control group T0 (p<0.001)

* p<0.001





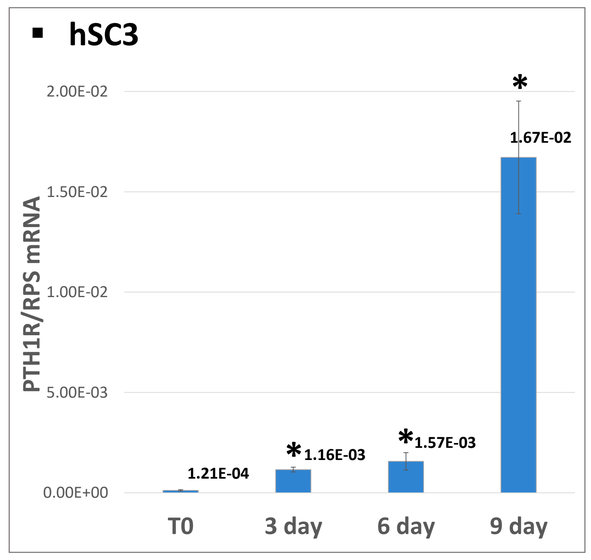


Fig 8. PTH1R gene analysis during myogenic differentiation of human satellite cell lines (hSC1, hSC2, hSC3) by real time quantitative TaqMan qPCR, normalized with housekeeping gene RPS18 (Average ± SD, Significance VS. T0)

CONCLUSION

The results have shown the successful isolation and characterization of cultured hSCs and the establishment of an *in vitro* myogenesis model, used for the PTH1R expression analysis. The major finding of the studies is detection of increase in expression of the PTH1R gene during myogenic differentiation, suggesting the possible involvement of PTH1R in myopathies related to hypoparathyroidism.

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