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CaSR PROTEIN EXPRESSION ANALYSIS IN STEM CELLS DERIVED FROM HUMAN SKELETAL MUSCLE AND HUMAN ADIPOSE TISSUE

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INTRODUCTION

CaSR is expressed in many tissues other than the parathyroid gland, where it plays various important roles. The contractile property of muscle fiber depends mainly on Ca^{2+}. Since bone, the main depot of Ca^{2+} in body, is present in close proximity to skeletal muscle, it is plausible the presence of CaSR in this tissue for modulating muscle activities such as Ca^{2+}-triggered muscle contraction, growth and differentiation. Satellite cells (SCs) are mononuclear skeletal muscle stem cells, which undergo myogenesis to repair skeletal muscle. There are many pathological conditions associated with failure of SCs activation. In this work, we have analyzed the *in vitro* expression of CaSR in isolated SCs and during myogenenesis. Since, SCs account only 2 to 7% of adult skeletal muscle, we have also analyzed the expression of CaSR in another source of mesenchymal stem cells i. e. human adipose tissue-derived stem cells (AMSCs), which can be obtained in large quantity by liposuction, to be used as another model to study *in vitro* myogenesis.

MATERIALS AND METHODS

Human skeletal muscle and adipose tissue biopsies were obtained from the patients undergoing surgery, after signed an informed consent approved by the Local Ethical Committee. The biopsies were minced and treated with collagenase for 3h at 37°C, and subsequently mechanically dispersed. The primary cells obtained were cultured in growth medium at 37°C and 5% CO_2, and characterization of obtained cell line was performed by flow cytometry with a CyFlowSpace cytometer (System Partec) equipped with FCS Express 6 software (De novo software, USA) for the expression of main nuclear transcription factor, PaX7 in SCs and, molecular markers CD90, CD105, CD44, CD34 and CD45 in AMSCs. Afterward, we have performed cytoflurometric analysis to detect CaSR protein expression in SCs and AMSCs using two types of anti-CaSR antibodies i. e. polyclonal anti-CaSR antibody (Abcam: ab 137408) and monoclonal anti-CaSR antibody (Abcam: ab 19347). To study the expression of CaSR during myogenic differentiation, SCs were grown in differentiation medium (Promo Cell) for 7 days and characterized for the myogenic phenotype and expression of terminal differentiation marker Myosin Heavy Chain (MHC) using immunofluorescence (IF) observation in Laser Scanning Confocal Microscopy (LSCM) using a LSM 510 Meta microscope (Carl Zeiss). Afterward, we have grown SCs for different time points (T_0, 4, 6, 8 days) in myogenic differentiation medium and performed IF analysis using anti-CaSR antibody 137408 to detect variation in expression of CaSR during *in vitro* myogenesis.

RESULTS

We have isolated and established the primary culture of SCs and AMSCs from human skeletal muscle and adipose tissue biopsies, respectively, to be used for our experiments (**Fig. 1**). Cytoflurometric analysis in SCs has shown that 98.29% of the cells are positive for expression of PAX 7 (**Fig. 2 A**), subsequently the same analysis to detect presence of CaSR in SCs has shown that 94.51% and 97.08 % of the cells are positive for CaSR expression with anti-CaSR antibody (137408) and (19347), respectively (**Fig. 3 A, B**). AMSCs have been characterized for the expression of mesenchymal stem cell markers, CD90 (99.74 %), CD105 (92.85 %), CD44 (92.85 %) CD34 (1.48%), CD45 (0.16%) with cytoflurometric analysis (**Fig. 2 B-F**). Subsequently, the same analysis to detect presence of CaSR in AMSCs has shown that 97.65 % and 98.94 % of cells are positive for CaSR expression with anti-CaSR antibody (137408) and (19347), respectively (**Fig. 3 C, D**). SCs after 7 days of myogenic induction have shown the myogenic phenotype i. e. multinucleated cell referred as myotubes, observed using phase contrast microscopy, and the IF staining has permitted the MHC protein in observed multinucleated cells (**Fig. 4**). IF analysis for the expression of CaSR in SCs at different time points (T_0, 4, 6, 8 days) of myogenic differentiation has shown high expression of the protein after 4 days of myogenic induction (**Fig. 5**).

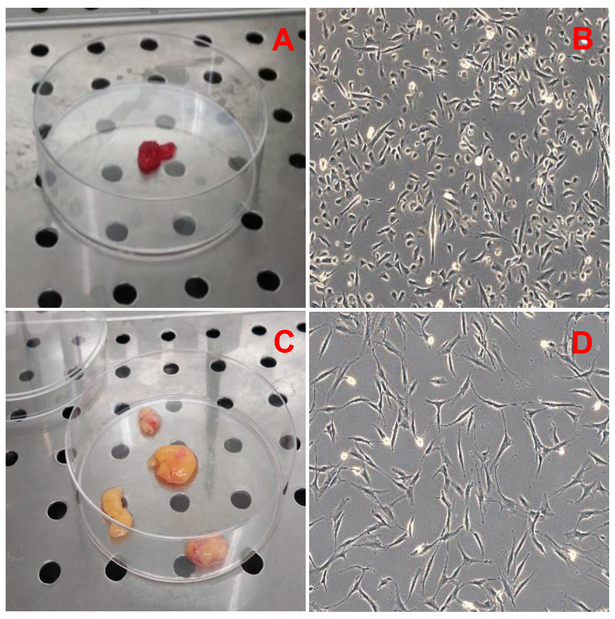

Fig. 1 Human skeletal muscle biopsy (A), Representative image of primary culture of SCs in phase contrast microscopy, objective 10x (B), Human adipose tissue biopsy (C), Representative image of primary culture of AMSCs in phase contrast microscopy, objective 10x (D).

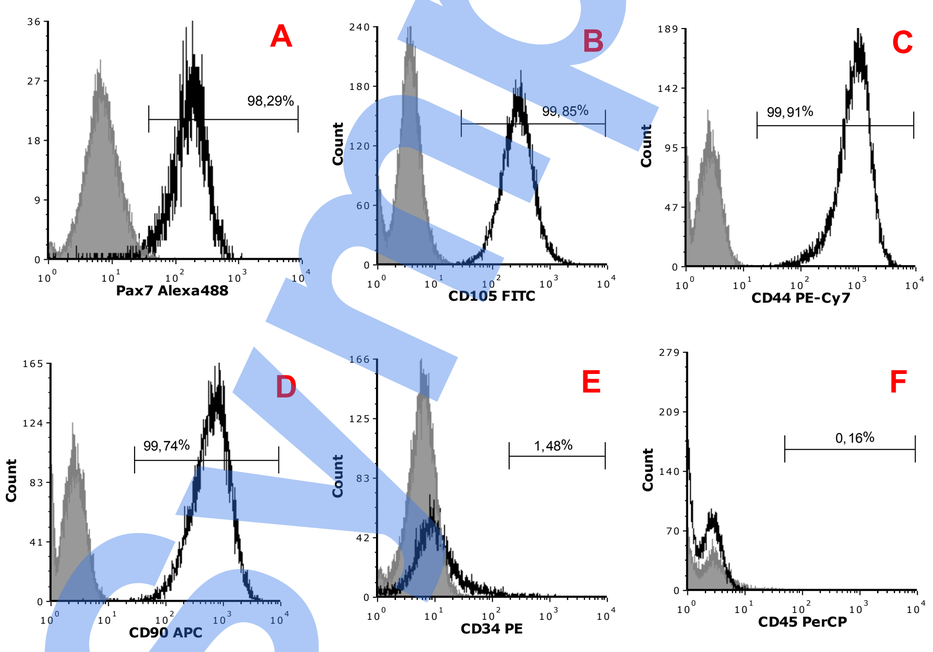

Fig. 2 Phenotype characterization of SCs (A) and AMSCs (B-F) by flow cytometry analysis.

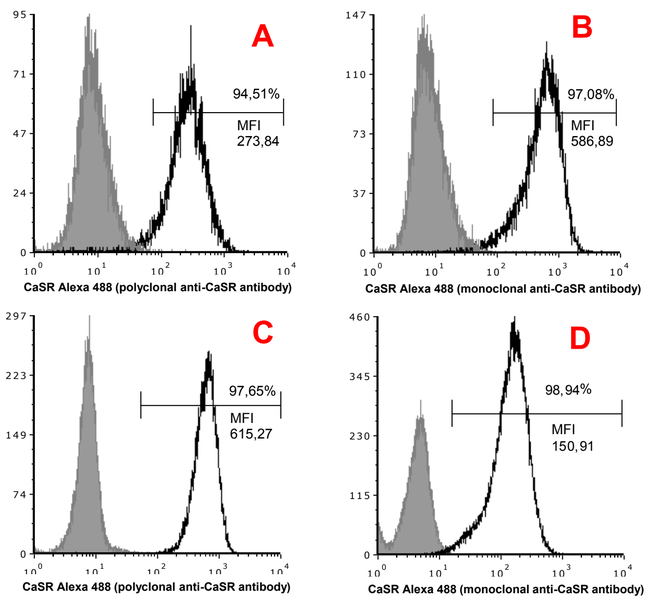

Fig. 3 Flow cytometry analysis representing the expression of CaSR using anti-CaSR polyclonal (Abcam: 137408) and monoclonal (Abcam: 19347) antibodies in SCs (A, B) and in AMSCs (C, D).

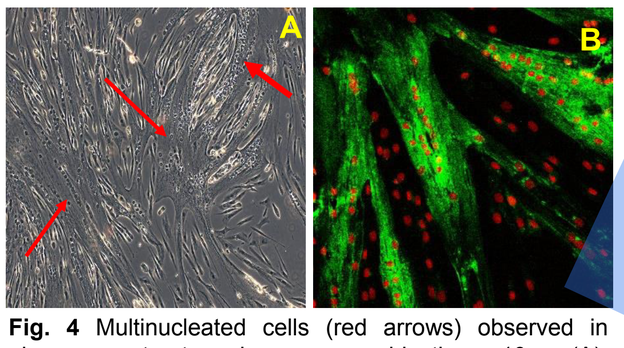

Fig. 4 Multinucleated cells (red arrows) observed in phase contrast microscopy, objective 10x (A), Representative image in LSCM of the MHC protein (green), nuclei (red/orange), objective 20x (B) after 7 days of myogenic induction.

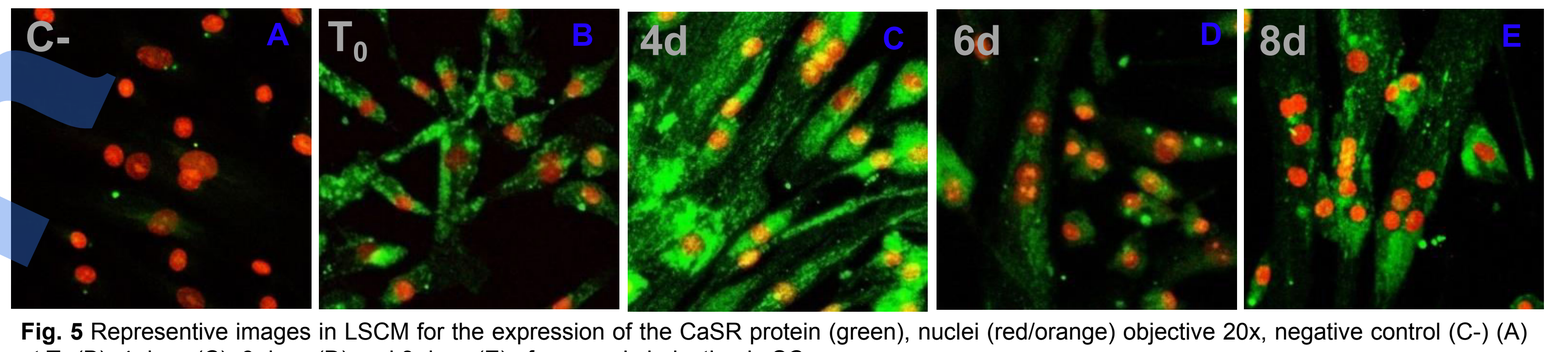

Fig. 5 Representive images in LSCM for the expression of the CaSR protein (green), nuclei (red/orange) objective 20x, negative control (C-) (A) at T_0 (B), 4 days (C), 6 days (D) and 8 days (E) of myogenic induction in SCs.

CONCLUSION

Our results have demonstrated the successful isolation and characterization of SCs and AMSCs derived from human skeletal muscle and adipose tissue biopsies, respectively. The results of cytoflurometric analysis have shown the presence of CaSR protein in our proposed models, SCs and AMSCs using two types of anti-CaSR antibody targeted against a region within amino acids 151-376 (Abcam: ab 137408) and 214-235 (Abcam: ab 19347) of human CaSR. Moreover, our results of *in vitro* myogenesis have demonstrated that SCs isolated from human skeletal muscle biopsies can be differentiated into myotubes, as shown by the presence of MHC and multinucleated cells, suggesting the suitability of our model for further experiments. Preliminary data have shown high presence of this receptor in SCs after 4 days of myogenic differentiation. Studies are in progress to analyze the expression of CaSR in our proposed models using calcium binding assay, secondary messenger analysis and western blot.

ACKNOWLEDGEMENT

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