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CALCIUM-SENSING RECEPTOR EXPRESSION ANALYSIS IN HUMAN SKELETAL MUSCLE TISSUE AND SATELLITE CELLS

Preeti Sharma[1], Cecilia Romagnoli[1], Roberto Zonefrati[1], Alessandra Aldinucci[2], Marco Innocenti[1], Luisella Cianferotti[1], Enikö Kallay[3] and Maria Luisa Brandi[1]

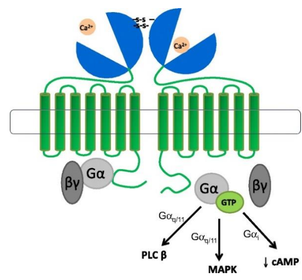
[1] Department of Surgery and Translational Medicine, University of Florence, Florence, Italy

[2] Department of Neurofarba, University of Florence, Florence, Italy

[3] Department of Pathophysiology and Allergy Research, Medical University of Vienna, Austria

INTRODUCTION

Calcium-sensing receptor (CaSR), a G protein-coupled receptor, is expressed primarily within parathyroid glands and it acts as the main systemic regulator of calcium homeostasis. Ca^{2+} is the main physiological ligand of CaSR. CaSR is also expressed in many other tissues, where it plays various important roles including gene expression, proliferation and differentiation, thus considered as an important molecule in physiology [1]. As per our knowledge, there is no report about CaSR expression in human skeletal muscles. Ca^{2+} is playing important roles in skeletal muscle physiology such as contraction, growth and differentiation. Additionally, the bone, which is the main depot of Ca^{2+} in the body, is present in close proximity to skeletal muscles. These facts lead us to hypothesize 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 stem cells of skeletal muscle, which in response to exercise and injury get activated and undergo myogenesis; in turn repair skeletal muscles [2]. Failure of SCs to undergo myogenesis has been observed in many muscular diseases. In this work, we have analyzed the *in vitro* expression of CaSR in human skeletal muscle tissue, in their isolated SCs and during myogenesis.



MATERIALS AND METHODS

Human skeletal muscle 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 and subsequently mechanically dispersed. The primary cells obtained were cultured in growth medium (PromoCell) at 37°C and 5% CO_2, and characterization of obtained SCs were done for the expression of main nuclear transcription factor, *PaX7* gene and protein by qualitative PCR and flow cytometry with a CyFlowSpace cytometer (System Partec) equipped with FCS Express 6 software (De novo software, USA), respectively. To establish a model of *in vitro myogenesis*, 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) by immunofluorescence (IF) observation in Laser Scanning Confocal Microscopy (LSCM) using a LSM 510 Meta microscope (Carl Zeiss). Afterward, the analysis of *CaSR* gene expression was performed with qualitative PCR using couples of primers targeting exons 2/3, exons 4/5 and exons 6/7 of human CaSR, using parathyroid cells as positive control, after verification of expression of housekeeping gene β-actin, in cDNA samples of human skeletal muscle tissue, SCs and in myogenically differentiated SCs. Subsequently, the cytoflurometric analysis was performed to detect CaSR protein expression in SCs using two types of anti-CaSR antibodies, i. e. Abcam polyclonal (ab 137408) and monoclonal (ab 19347). Moreover, to detect variation in expression of CaSR during *in vitro* myogenesis, the SCs were grown for different time points (T_0, 4, 6, 8 days) in myogenic differentiation medium and performed IF analysis using anti-CaSR antibody ab137408. The IF analysis in deparaffinized human skeletal muscle tissue sections was performed using non-commercial anti-CaSR antibodies.

RESULTS

We have cultured and characterized the SCs isolated from healthy human skeletal muscle biopsies (**Fig. 1**). We have established an *in vitro* model of myogenesis using SCs (**Fig. 2**). The cytoflurometric 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 (ab 137408) and (ab 19347), respectively (**Fig. 3**). Subsequently, IF analysis for the expression of CaSR in SCs at different time points (T_0, 4, 6, 8 days) of myogenic differentiation using anti-CaSR antibody (ab 137408), has shown high expression of the protein after 4 days of myogenic induction (**Fig. 4**). In order to analyze the unspecificity of our commercial anti-CaSR antibodies that we have used, we have performed cytoflurometric analysis in HEK293 cells with same antibodies, which is known for not expressing CaSR. The cytoflurometric analysis of CaSR in HEK293 cells has shown that the commercial anti-CaSR antibodies (Abcam: 137408 and 19347) have unspecific binding, and are not suitable for CaSR protein analysis (**Fig. 5**). Our results of the qualitative PCR analysis have demonstrated the absence of *CaSR* gene expression in human skeletal muscle tissue, their isolated SCs and during *in vitro* myogenesis (**Fig. 6 & 7**). Additionally, our results of immunostaining using non-commercial monoclonal and polyclonal anti-CaSR antibodies have shown absence of CaSR protein in skeletal muscle tissue sections (**Fig. 8).**

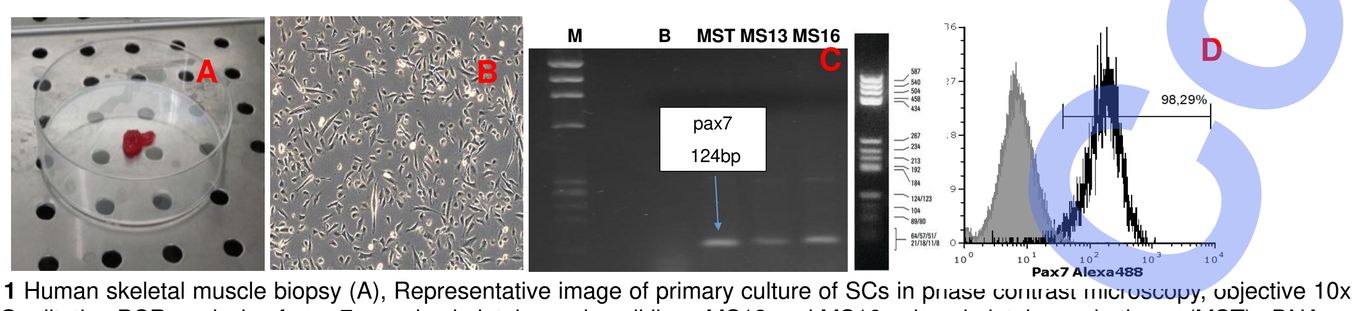


Fig. 1 Human skeletal muscle biopsy (A), Representative image of primary culture of SCs in phase contrast microscopy, objective 10x (B), Qualitative PCR analysis of *pax7* gene in skeletal muscle cell lines MS13 and MS16 using skeletal muscle tissue (MST) cDNA as positive control (C), Phenotype characterization of SCs using Flow cytometry analysis for the presence of Pax 7 protein (D).

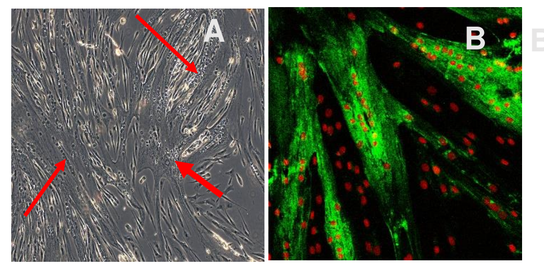


Fig. 2 Multinucleated cells (red arrows) observed in phase contrast microscopy, objective 10x (A), Representative image in LSCM of the MHC protein (green), nuclei (red/orange), objective 20x (B) after 7 days of myogenic induction.

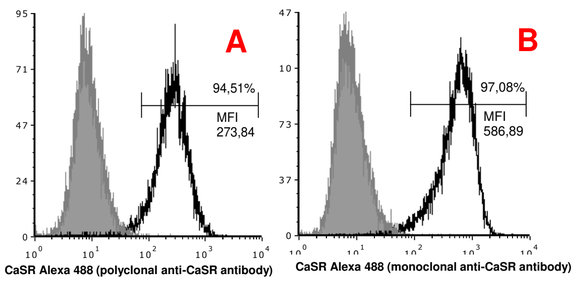


Fig.3 Flow cytometry analysis representing the expression of CaSR using anti-CaSR polyclonal (Abcam: 137408) and monoclonal (Abcam: 19347) antibodies in SCs (A, B)).

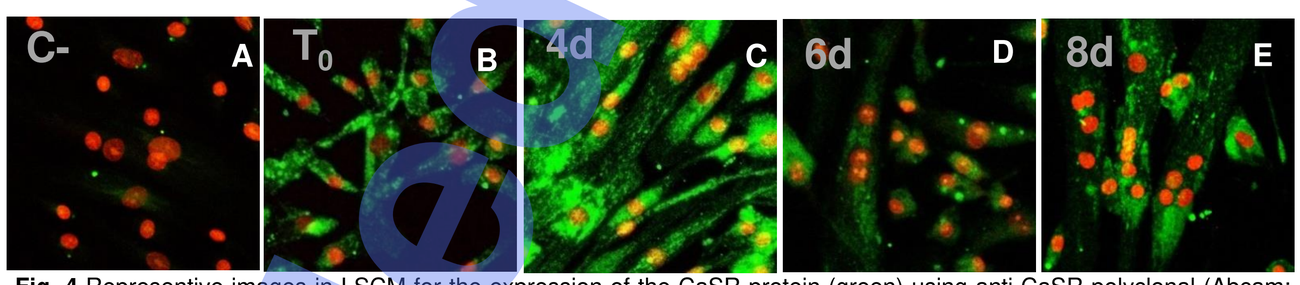


Fig. 4 Representine images in LSCM for the expression of the CaSR protein (green) using anti-CaSR polyclonal (Abcam: 137408), nuclei (red/orange) objective 20x, negative control (C-) (A) at T_0 (B), 4 days (C), 6 days (D) and 8 days (E) of myogenic induction in SCs.

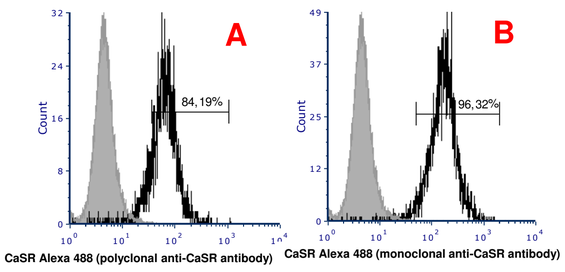


Fig.5 Flow cytometry analysis representing the unspecific binding of anti-CaSR polyclonal (Abcam: 137408) and monoclonal (Abcam: 19347) antibodies in HEK293 cells (A, B)).

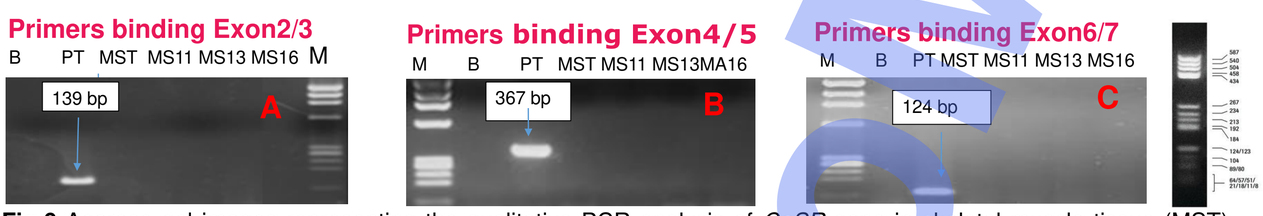


Fig.6 Agarose gel images representing the qualitative PCR analysis of *CaSR* gene in skeletal muscle tissue (MST), and in skeletal muscle cell lines MS11, MS13 and MS16 with parathyroid (PT) cDNA as positive control using primers binding to exon2/3 (A), exon4/5 (B) and exon6/7 (C) of human CaSR.

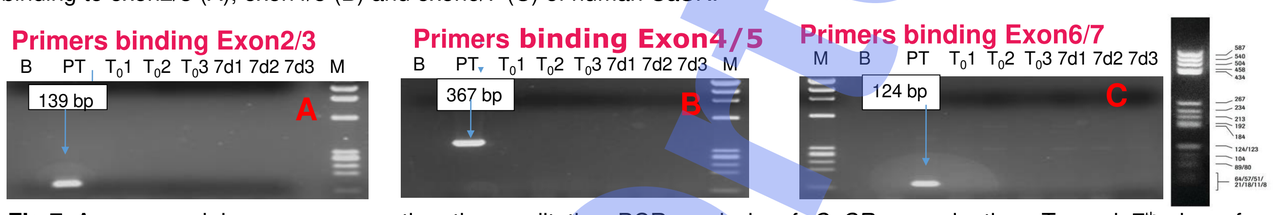


Fig.7 Agarose gel images representing the qualitative PCR analysis of *CaSR* gene in time To and 7th day of myogenically differeciated SCs in three replicates with parathyroid (PT) cDNA as positive control using primers binding to exon2/3 (A), exon4/5 (B) and exon6/7 (C) of human CaSR.

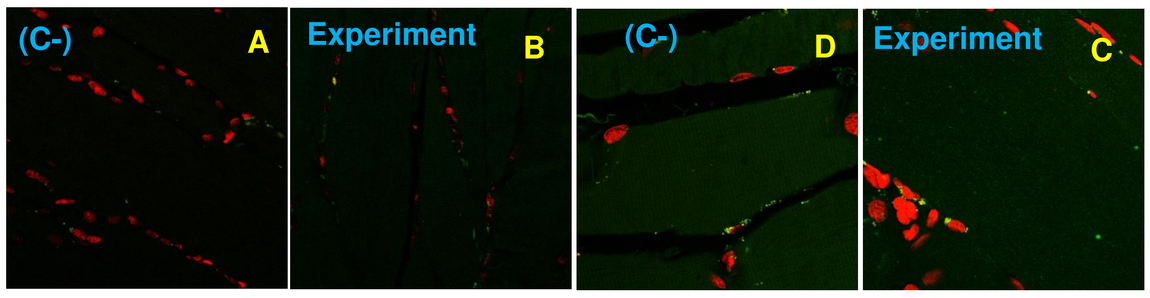


Fig. 8 Representative images in LSCM for the expression of the CaSR protein (green) and nuclei (red) using anti-CaSR monoclonal, objective 40x, negative control (C-) (A), experiment (B), using anti-CaSR polyclonal, objective 63x, negative control (C-) (C), experiment (D).

CONCLUSION

Our results have demonstrated the successful isolation, characterization and establishment of model of *in vitro* myogenesis using SCs derived from human skeletal muscle, for further experiments. Our results have shown the absence of *CaSR* gene in human skeletal muscle tissue, in their isolated SC and during in vitro myogenesis. Additionally, our results have also shown the absence of CaSR protein in human skeletal muscle tissue. Studies are in progress to analyze the expression of CaSR protein in SCs and during *in vitro myogenesis.* Our findings, support the fact that probably CaSR is not having physiological importance in skeletal muscle, thus does not express in this tissue.

ACKNOWLEDGEMENT

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