Unstable expression of GPRC6A in human pancreatic β-cells

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INTRODUCTION

GPRC6A is a widely expressed seven-transmembrane G-protein-coupled receptor mediating L-α-amino acids signaling (mainly, arginine, lysine, and ornithine). There is increasing evidence that GPRC6A is involved in the regulation of inflammation, metabolism and endocrine functions [1].
Recently it has been proposed as a receptor for the bone-derived hormone osteocalcin (OC) [2]. By interacting with GPRC6A, in β-cells, OC is thought to modulate the glucose-induced insulin secretion [3] However, GPRC6A functions have been studied only in rodents or in rodents-derived cells. Aim of this study was to characterize the GPRC6A expression in different culture conditions in 1.1B4 cells, a human model of pancreatic β-cells [4].

METHODS

1.1B4 cells were obtained from Sigma-Aldrich and cultured in RPMI-1640 medium supplemented with 10% FBS.
To evaluate GPRC6A expression, cells were seeded at different densities, from $2 \cdot 10^3$ to $4 \cdot 10^4/cm^2$ and harvested every day, over 7 days.
At each time-point, total RNA was extracted using the the PureLink® RNA Mini Kit (ThermoFisher Scientific), reverse transcribed to cDNA (iScript cDNA Synthesis Kit, Bio-Rad Laboratories) and amplified through RT-PCR by means of GPRC6A specific primers. In parallel GPRC6A protein expression in cell lysates was evaluated by Western Blot, using a rabbit anti-human GPRC6A antibody (OriGene Technologies).
In order to investigate the possibility to overexpress GPRC6A, and the transfection efficiency, 1.1B4 cells were transfected using TurboFect transfection reagent (Thermo Fisher Scientific) according to the manufacturer's recommendations. The cells were seeded in six-well plates at a density of $3 \cdot 10^3/cm^2$ for 48h, starved for 24h and then transfected with pCMV6-neoGPRC6A plasmid (4 µg/well, OriGene Technologies). The overexpression of the receptor was evaluated by means of RT-PCR and Western Blot, from 24h to 144h.

RESULTS

Fig.1

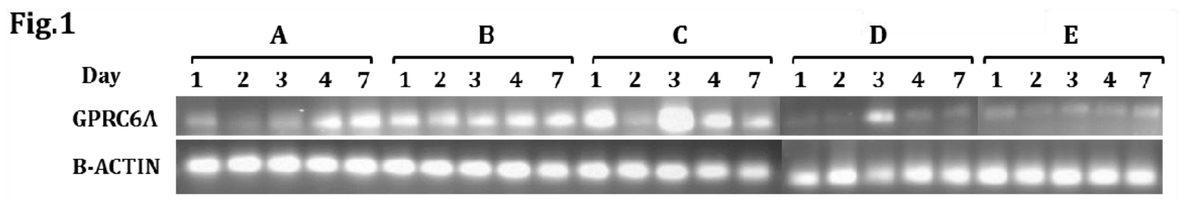


Fig.2

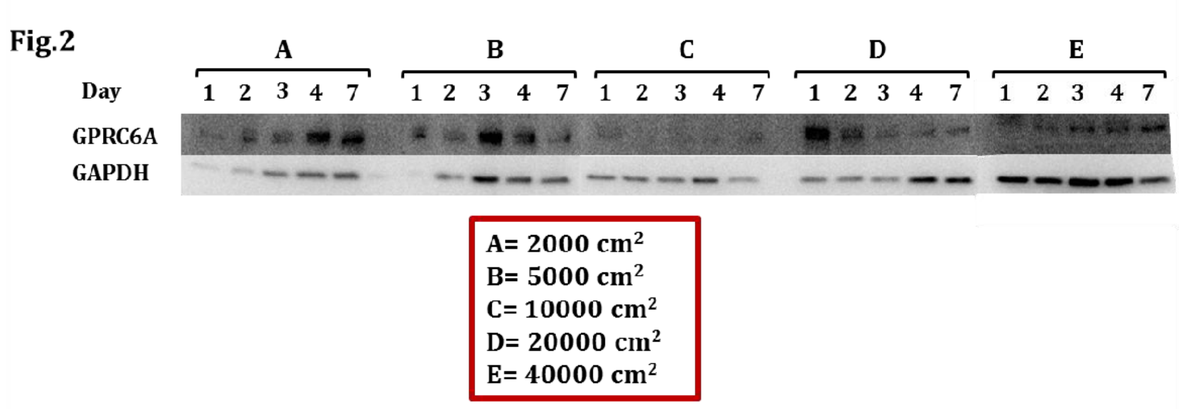


Fig.3

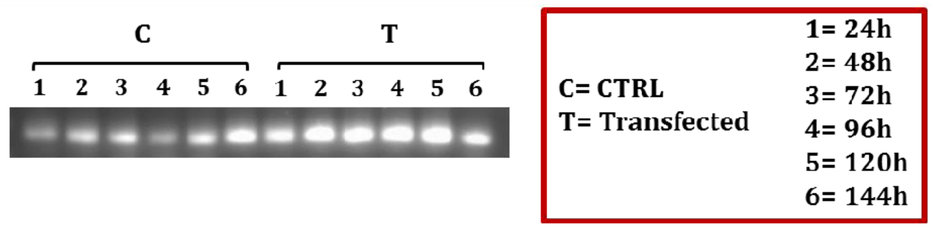
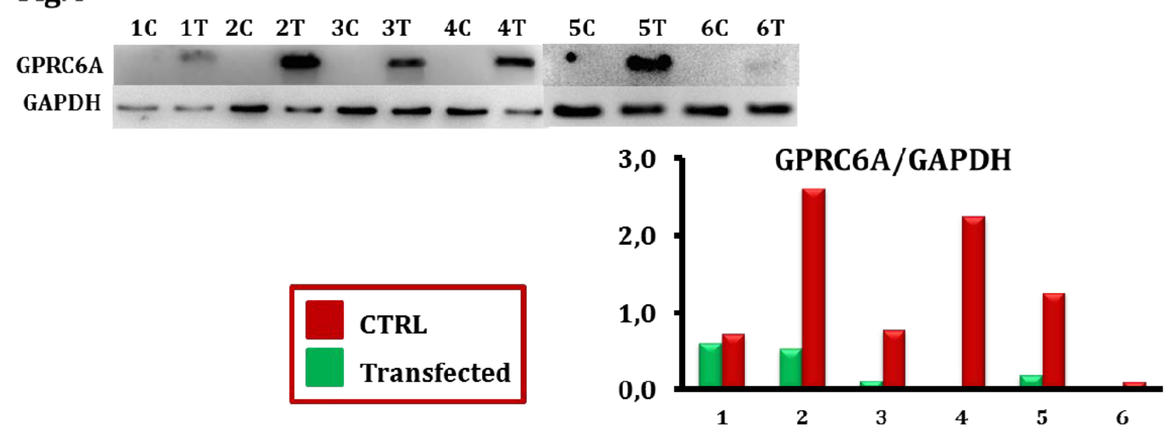


Fig.4



At low cell densities ($2 \cdot 10^3$ to $5 \cdot 10^3$ cells/cm^2, conditions A and B) both PCR (Fig. 1) and western blot (Fig. 2) there was a time-dependent increase in GPRC6A expression, which remained, then, stable from day 4 to day 7. Instead, at cell densities equal to or higher than $1 \cdot 10^4/cm^2$ (conditions C, D and E) mRNA expression of the receptor was quantitatively decreased compared to lower cell densities. Moreover, especially at $1 \cdot 10^4/cm^2$, gene expression was highly fluctuating results. The fluctuating behavior was also evident in protein expression analysis although the expression profile evidently differed form those obtained by RT-PCR. These cell density- and time in culture-independent fluctuations in GPRC6A mRNA and protein expression in 1.1B4 cells make functional studies, on this receptor, impossible.
In order to obtain a quantitatively stable expression of GPRC6A, aimed at investigate its function in 1.1B4 pancreatic β-cells, cells were transiently transfected with pCMV6-neoGPRC6A plasmid. Under this condition GPRC6A mRNA and protein expression were followed up to 6 days. Figures 3 an 4 shows that, compared to control cultures, GPRC6A expression remained stably high up to 120h in terms of both mRNA and protein.

CONCLUSIONS

Recent studies have revealed the role of GPRC6A in the involvement of OC in glucose metabolism [2].
In this study we evaluated the expression of GPRC6A in a human model of pancreatic β-cell.
Our data demonstrates an intrinsic instability of these cells in terms of GPRC6A expression. In order to study the functions of GPRC6A and its putative interactions with OC, its overexpression is needed.

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