Filamin-A is involved in stabilization, signal transduction and angiogenesis regulation mediated by Somatostatin Receptor 2 (SST2) in pancreatic neuroendocrine tumors (P-NETs)

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Somatostatin (SS) is an ubiquitous peptide that physiologically inhibits hormone secretion and cell proliferation in neuroendocrine cells (1). These effects are mediated by five receptor subtypes (SSTRs-5) belonging to the G protein coupled receptors superfamily (GPCRs) that differ in tissue distribution, affinity to ligands and regulation (2, 3). Somatostatin receptor type 2 (SST2) is the main pharmacological target of long-acting somatostatin analogues (SSA) widely used in patients with pancreatic neuroendocrine tumors (P-NET) (1, 4), this treatment being ineffective in a subset of patients (5), but the mechanisms involved in the resistance are still unknown. Several studies demonstrated that GPCRs expression and signalling are mediated by different cytoskeletal proteins, including filamin A (FLNA) (6, 7). FLNA is an ubiquitous actin binding protein, that acts as a molecular scaffold for several proteins, including transmembrane proteins and signalling molecules. Recently, FLNA-SST2 interaction has been found to be a critical role for SST2 stabilization and cell signalling (8, 9). Moreover, the involvement of FLNA in angiogenesis has been suggested as a target for neo-vascular cancer therapy in vitro. In fact, a positive relationship between FLNA and vascular endothelial growth factor (VEGF) was found in patients with lung cancer (10), suggesting that FLNA is implicated in angiogenesis through links with VEGF. Interestingly, it has been demonstrated that VEGF pathway is overexpressed in neuroendocrine tumors (11), this pathway being inhibited by somatostatin analogues (12).

The aim of the present study was to investigate the role of FLNA in the regulation of SST2 stabilization, signalizing and angiogenesis in pancreatic neuroendocrine tumours.

MATERIAL AND METHODS

- Cell culture and silencing: Short interfering RNA (siRNA) were purchased from Invitrogen. QCG-2 cells were transfected with 200 pmol of siFLNA, or negative control siRNA (C- siRNA) for 72h, using Lipofectamine 2000 according to the instruction of the manufacturer.
- cAMP assay: QCG-2 cells transfected with FLNA siRNA or C-siRNA were stimulated for 0, 5, 10 and 30 minutes with 10 uM forskolin with or without the SST2 selective agonist BIM23180 (10 nM) for 30 minutes and cellular cAMP was measured by enzymatic immunoassay (Promega, Madison, WI, USA).
- Immunohistochemistry: was performed on sections from 29 P-NETs retrieved from the archives of Pathology Unit of IRCCS Humanitas Research Hospital, Rozzano, Milan Italy. After dewaxing in xylene and rehydrating in ethanol, the sections were pretreated in a water bath set to 98°C in 0.01 M citrate buffer for 25 minutes. FLNA antibody (Millipore, 1:5000 dilution) and SST2 (UMB-1; Abcam; 1:200 dilution) was used, and antigen-retrieval was performed with the MACH1 universal polymer detection kit (Biocare Medical).
- VEGF secretion study: silenced or non silenced QCG-2 cells were treated with or without BIM23180 (10 nM) in serum free RPMI-1049 medium for 72h at 37°C. Collected supernatants were used to measured VEGF concentration with ELISA kit (Invitrogen, Camarillo, CA, USA), according to manufacturer instructions.
- Western Blot Analysis: All samples were separated on SDS-PAGE, and the proteins were detected by Western Blotting using antibodies against FLNA (AlNova), SST2 (Santa Cruz), GAPDH (Amerham), CD1 (Millipore), pERK1/2 and ERK1/2 (Cell Signaling). Immune detection was performed using Odyssey Imaging system (LI-COR).

CONCLUSIONS

- FLNA is not required for basal SST2 expression but it stabilizes the receptor expression after long-term agonist stimulation.
- FLNA is required for SST2-mediated cell proliferation and cAMP accumulation inhibition.
- FLNA is crucial for SST2-mediated angiogenesis inhibition

REFERENCES