High-resolution spatiotemporal analysis of somatostatin receptor type 2 (SSTR2) – Filamin A (FLNA) interaction by single-molecule imaging

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SSTR2 is a G₂ coupled receptor used as pharmacological target for GH-secreting pituitary adenomas treatment, however a subset of patients displays resistance to somatostatin analogues. We recently demonstrated that the cytoskeletal protein FLNA plays an essential role in tumor responsiveness by regulating SSTR2 signaling and stabilization after prolonged stimulation in human and rat somatotroph cells.

The aim of this study was to follow the spatiotemporal behavior of SSTR2-FLNA complexes in real time in living cells by high resolution single-molecule imaging. In particular we wanted to investigate the presence of a spatial distribution of FLNA-SSTR2 complexes at the plasma membrane, to estimate SSTR2 lateral mobility and the involvement of FLNA in regulating this biological phenomenon, and eventually elucidate a possible role of SSTR2 in FLNA clustering organization and internalization after ligand binding.

### Materials and Methods

- **Cloning:** Snap-tagged SSTR2 was obtained by fusing a SNAP tag at the C-terminal of a WT-SSTR2 in a pcDNA3 vector. SNAP-tagged FLNA was generated by replacing GISP with a CLIP tag at the first hinge region of a FLNA-GISP plasmid.
- **Transient cell transfection:** Transient transfections of CHO, A7, M2 cells were performed using the transfection reagent Lipofectamine2000 according to the instruction of the manufacturer; HEK293A cells transient transfection (48 hours) have been performed with Efficiency.
- **Measurements of cAMP concentration:** Cyclic and transient experiments to determine cAMP levels were performed in HEK293/1A cells co-transfected with SNAP-tagged SSTR2 or WT-SSTR2 together with cAMP sensor.
- **Single-Molecule Imaging:** CHO, A7, M2 cells transiently expressing SNAP/CLIP proteins were grown on coverslips at the cell density of 3,000 cells/well. After 4 hours of transfection cells were labeled with fluorescent dyes (LiM) and imaged at a Nikon TIRF microscope equipped with an EM-CCD camera and temperature controller. The laser powers were set at 30%.
- **Analysis of receptor mobility:** The distributions of SSTR2 diffusion coefficients were calculated by u-track algorithm implemented in Matlab.

### Functional characterization

- **SNAP-SSTR2:** Cell-surface localization
- **CLIP-FLNA:** SNAP-FNA, SNAP-FLNA co-localization with actin

### Results

**FLNA-SSTR2 spatial arrangement at the plasma membrane**

SSTR2 is widely distributed at the cell surface under basal condition whereas upon ligand binding it associates in clusters organized on FLNA fibers.

**SSTR2 lateral mobility: effect of agonist on receptor speed**

The selective SSTR2 agonist increases the immobile receptors population.

**Dynamic visualization of SSTR2 – FLNA interactions**

SSTR2-FLNA interactions are extremely dynamic and transient under basal condition, whereas after receptor stimulation static SSTR2s localized along FLNA fibers undergo internalization...

...suggesting a role of FLNA in receptor endocytosis.

### Conclusions

- Dynamic SSTR2-FLNA interactions become more static and stable upon SSTR2 agonist incubation.
- The SSTR2 agonist increases the fraction of immobile receptors which co-localize with FLNA.
- SSTR2 clusters and internalizes along FLNA fibers in stimulated cells suggesting a possible role of FLNA in receptor endocytosis.