Agonist-Selective Phosphorylation Of The Human sst3 Somatostatin Receptor Determined By Phosphosite-specific Antibodies.

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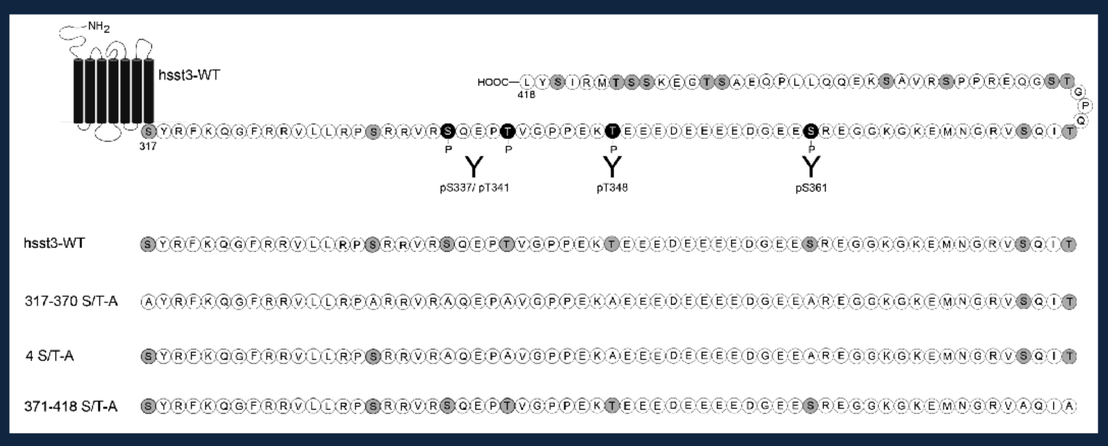
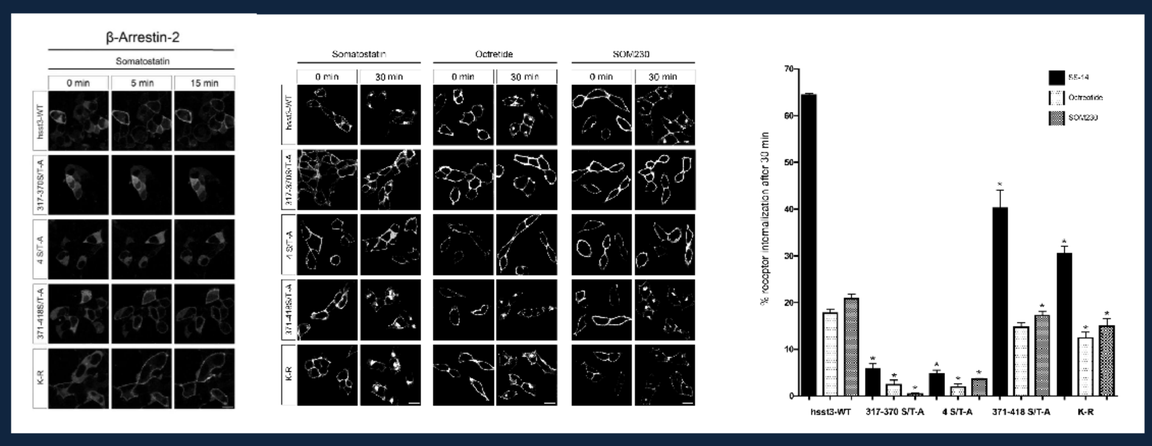
The human somatostatin receptor 3 (hsst3) is expressed in about 50 % of all neuroendocrine tumors. The sst3 receptor is unique among somatostatin receptors which can initiate apoptosis of tumor cells through activation of the tumor suppressor p53. Furthermore, treatment of the sst3 receptor with somatostatin or stable somatostatin analogs such as octreotide or pasireotide can inhibited tumor cell proliferation. However, at present little is known about the agonist-induced regulation of the human sst3 receptor. We have generated a series of phosphorylation-deficient mutants of the receptor and determined important sites for agonist-induced internalization. Based of this information we generated phosphosite-specific antibodies for the carboxyl-terminal serine 337, threonine 341, threonine 348 and serine 361, which enabled us to investigate the temporal patterns of sst3 phosphorylation and dephosphorylation. Here we demonstrate that pasireotide and octreotide were not able to promote a phosphorylation to the same extent as natural somatostatin. Similar the sst3-selective ligand L-796/778 did not promote any detectable phosphorylation or internalization. We also show that sst3 phosphorylation occurred within minutes, whereas dephosphorylation and recycling of the sst3 receptor occurred at a considerably slower rat. We also identify G protein-coupled receptor kinases 2 and 3 (GRK2/3) and protein phosphatase 1 (PP1) as key regulators of sst3 phosphorylation and dephosphorylation.

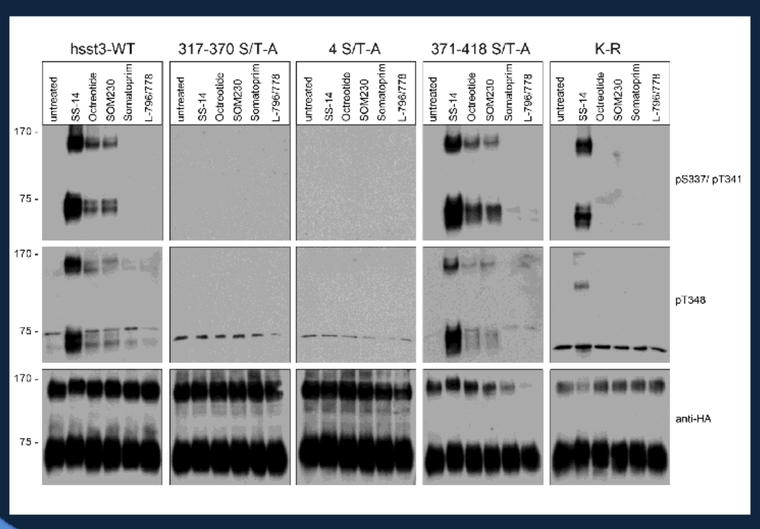
Human sst3 receptors with mutations of phosphorylations sites



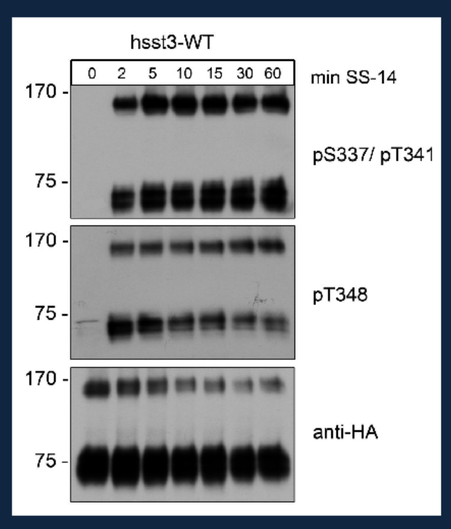
SS-14 induces β-Arrestin2 recruitment and leads to the strongest internalization



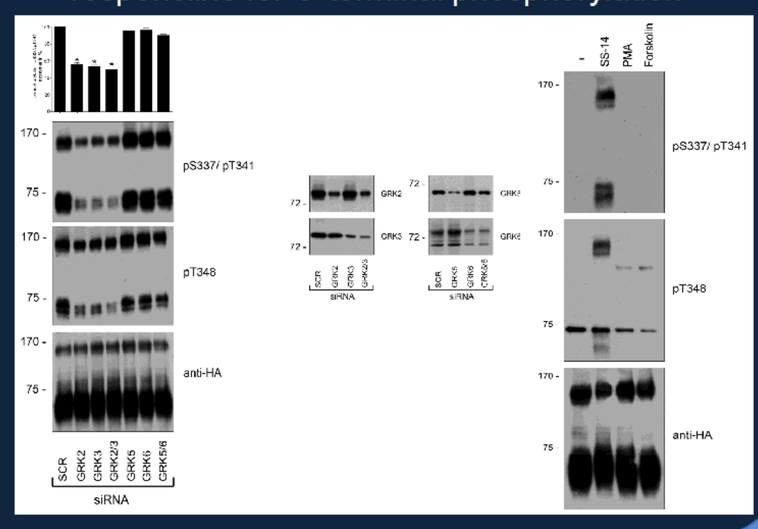
SS-14 as full agonist shows intense phosphorylation



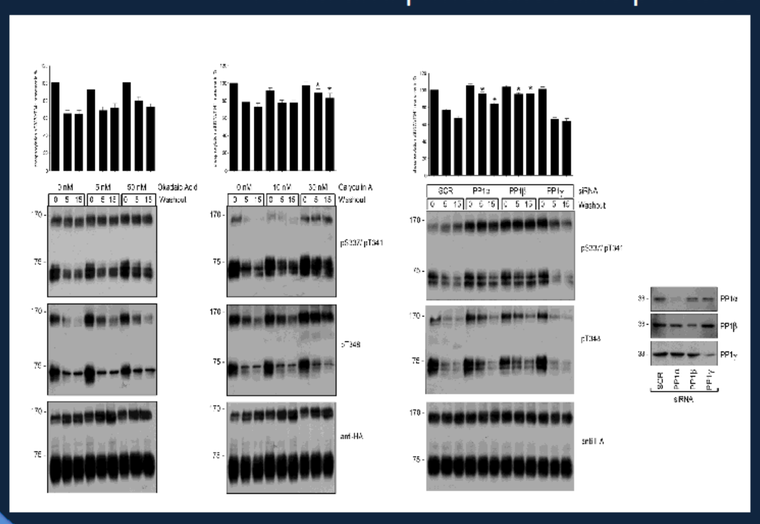
Phosphorylation kinetic



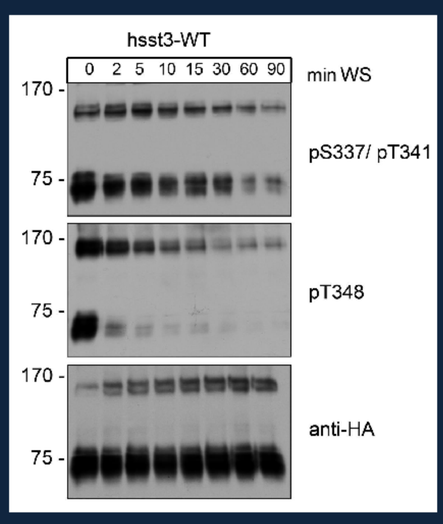
In homologous activation GRK2 and 3 are responsible for C-terminal phosphorylation



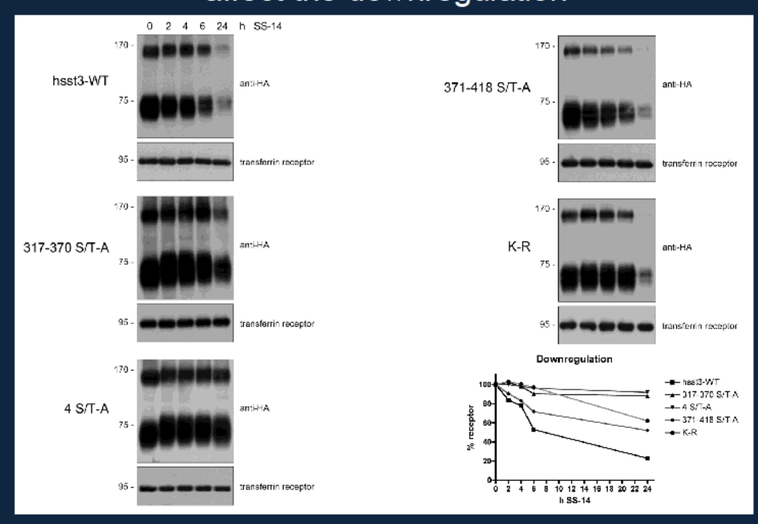
Only Calyculin A prevents dephosphorylation PP1α and β mediates this process



Dephosphorylation kinetic



Mutations of phosphorylation and lysine sites affect the downregulation



Conclusion

We have generated phosphosite-specific antibodies for the C-terminal serine 337, threonine 341, threonine 348 and serine 361.
These four phosphorylation sites are essentiell for the agonist-dependent β-Arrestin2 recruitment and internalization and they show different kinetics of phosphorylation and dephosphorylation. A siRNA-mediated knockdown revealed that GRK2 and 3 are responsible for phosphorylation and that PP1α and PP1β regulate the dephosphorylation.
Furthermore the hsst3-receptor is downregulated after a long-lasting stimulation with somatostatin. For this process conditions like phosphorylation and internalization have to be fulfil. Mutations of lysine sites delay the downregulation. So the ubiqutinylation of the receptor seems to play a critical role in the degradation of the recepor.

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