J. Biol. Res. 2011 n.1 - Vol. LXXXIV - Rubbettino - Soveria Mannelli

Somatostatin receptor 1,2 and 5 activation leads to C6 glioma growth arrest *in vitro* and *in vivo*: analysis of the intracellular pathways involved

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KEY WORDS: somatostatin, somatostatin receptors, gliomas.

Abstract

We report that somatostatin receptor (SSTR) 1,2 and 5 activation by selective agonists, causes C6 cell growth arrest through PTPη-dependent dephosphorylation of ERK1/2 in vitro and after xenografting in nude mice. Individual SSTR agonists displayed different efficacy and potency showing partial synergism by combined treatment. Since most tumor cells express multiple SSTRs, the activation of all the subtypes may grant a better control of cancer growth.

Introduction

Somatostatin (SST) exerts antiproliferative effects through the activation of 5 G protein-coupled receptors (SSTR1–5), often overexpressed in human tumors, including gliomas. Multiple intracellular pathways are involved in the antiproliferative effects of SST but the modulation of the phopshotyrosyne phosphatase (PTP) η activity was reported as a major mechanism in SST regulation of glioma growth. To deepen in the individual role of the different SSTR subtypes in the regulation of cell proliferation and the intracellular pathways involved, we used C6 rat glioma cells, natively expressing SSTR1, 2, 3 and 5, using the natural ligand (SST) and selective agonists in *in vitro* and *in vivo* experiments.

Materials and Methods

DNA synthesis was measured by [3H]-thymidine uptake. ERK1/2 activation was analyzed by western blot in the *in vitro* and by immunohistochemistry in the *in vivo* studies.

Results

Using selective agonists for SSTR1, 2, 3 and 5 in comparison with SST (active on all subtypes), we tested the role of different SSTRs on cell proliferation and activation of PTPη. SSTR1, 2 and 5 agonists (BIM-23745, BIM-23120, BIM-23206, respectively) were highly effective in reducing C6 cell growth, while SSTR3 agonist (L-796778) was devoid of antiproliferative effects in these cells. SST showed the highest potency and efficacy suggesting a cooperative activity of the single subtypes on the control of cell proliferation. As far as the selective compounds, SSTR5 agonist displayed the maximal efficacy and the SSTR2 agonist was the most potent compound [1]. To evaluate the intracellular mechanisms mediating the antiproliferative activity of individual SSTRs, we showed that the PTP inhibitor vanadate abolished the antiproliferative effects of all the agonists. SST and SSTR1, 2 and 5 agonists directly activated PTPη that, in turn, dephosphorylated and inactivated ERK1/2. The use of C6 glioma cells in *in vivo* experiments resembles with a sufficient approximation GBM growth, invasion, and neovascularization. We tested the effect of SST and selective SSTR agonists on the growth of C6 cells xenografted in nude mice. SST treatment (50μg, s.c. peritumoral, twice/day for 19 days) induced significant C6 tumor shrinkage, reaching a threefold reduction compared to control mice, without induction of toxic symptoms. Then we tested the ability of SST to affect tumor angiogenesis. C6-explanted tumors were analyzed by histological analysis (CD34 and CD31 positive cells), showing that SST caused a marked inhibition of angiogenesis as compared to untreated tumors. To delve deeper into the *in vivo* molecular mechanisms of SST activity, immunohistochemistry of C6 gliomas was carried out to evaluate the intratumoral levels of the active form ERK1/2 and the expression of p27Kip1, as indirect index of PTPη activation. SST treatment inhibited ERK1/2 activity and caused up-regulation of p27Kip1, as we observed *in vitro* [1]. Then, we analyzed the *in vivo* antitumoral effect of the individual SSTRs using SSTR1, 2, and 5 agonists. The activation of all the SSTR subtypes resulted effective in the inhibition of C6 glioma development, but the activation of SSTR5 resulted in a stronger effect than SSTR1 and 2, confirming the *in vitro* results. Conversely, SSTR1

and 2 agonists induced a more powerful inhibition of microvascular density formation [2]. The different efficacy of SSTR agonists on tumor growth inhibition and neovessel formation, suggests that the two processes might be caused by independent mechanisms. Finally, also the *in vivo* antitumor activity of each receptor agonist was mediated by a common intracellular pathway (inactivation of ERK1/2 and up-regulation of p27Kip1), as we observed *in vitro* [1].

Conclusions

The availability of specific SSTR agonists greatly improved the comprehension of the molecular effects of individual SSTRs. Since human tumors express multiple SSTRs, the characterization of the pattern of expression of each SSTR is the first approach for the diagnosis and therapy. Further studies on the characterization of the presence of specific receptor subtypes in cancer cells, and the mechanisms underlying the regulation of antiproliferative and pro-apoptotic pathways, may help to predict the potential efficacy of the pharmacological targeting of SSTRs. In our preclinical studies we report the activation of PTPη and the subsequent inhibition of ERK1/2, the molecular mechanism by which SST inhibits tumor growth through the activation of at least 3 SSTR subtypes (1, 2 and 5) although differences in the individual contribution of each receptor were detected. Moreover, the different efficacy of the SSTR selective agonists to inhibit tumor cell growth and neovessel formation suggests that to obtain maximal inhibitory effects the simultaneous activation of different subtypes may be required. Thus, the use of SST analogs combining multiple SSTR specificity may improve the potential of this therapeutic approach in antitumor strategy.

Grants: AIRC 2010 to TF.

References

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