CHARACTERIZATION OF ANTIPROLIFERATIVE EFFECTS OF SOMATOSTATIN IN GLIOMA CELL LINES

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The aim of this work has been the evaluation of the inhibitory effects of somatostatin on the proliferation of different brain tumor cell lines in vitro and in vivo after the inoculation in nude mice. Moreover we have investigated possible intracellular effectors for this activity.
The first task was to identify the expression of the sstr subtypes in post surgical specimen from brain tumours, obtained by the Department of Neurosurgery of the University of Genova (Prof. R. Spaziante). Up to date 23 gliomas have been analyzed, using the RT-PCR technique. All the gliomas (7 grade II, 6 grade III and 13 grade IV) showed the expression of at least 1 sstr mRNA. Sstr1 and sstr2 were the most represented in all the different classes of tumours analysed. Interestingly, the expression of sstr3, 4 and 5 in the gliomas was mainly limited to the low grade tumours (grade II) were they were expressed in about 60-70% of the samples compared to the 20-30% of the grade III and 20-40% of the grade IV. The expression of sstr1 and sstr2 was also confirmed in the human glioblastoma derived cell lines A172 and DB TRG, while only sstr2 was detected in the U87MG and U373 cell lines. The expression of the mRNA encoding sstr3, sstr4 or sstr5 was not detected in these cells. Also in the C6 rat glioma cell line, the sstr2 was the subtype but also sstr3 was expressed. These results, show that sstr1 and sstr2 are the main sstr subtypes expressed both in human gliomas and in the clonal cell lines. Then we have analyzed in the human glioblastoma cell lines available the antiproliferative effects of somatostatin and of some selective analogues able to selectively bind and activate specific somatostatin receptors (sstr). The cell lines analyzed were: A172, U87 MG, DB TRG, and U373 human glioblastoma and the C6 rat glioma cells.In all these cell lines we evaluated the effects of somatostatin on the basal and growth factor-stimulated cell proliferation, measured using the [^{3}H]-thymidine incorporation assay. From these experiments we identified three cell lines that are responsive to somatostatin, namely A172, U87 MG and C6, while the other two cell lines (U373 and DB TRG) are completely insensitive to the antiproliferative effects of the peptide. Somatostatin inhibited "basal" DNA synthesis (i.e. [^{3}H]-thymidine incorporation after 48 hrs. of serum starvation) in A172 cells. This effect was evident since, also in these experimental conditions, the cells showed a significant ERK1/2 activation and a still present proliferative activity.

Basic FGF (50ng/ml) stimulated A172 cell proliferation (+80% over basal) an effect also inhibited dose-dependently by the somatostatin treatment (10nM-10μM). Moreover, in bFGF-stimulated conditions, sstr1 and sstr2 selective somatostatin analogs BIM 23745 and BIM 23120 inhibited A172 cell proliferation, although the SSTR1 agonist was significantly more powerful.
A more powerful inhibition was observed in U87MG cells stimulated with IGF-1 (50 ng/ml). In these cells the growth factor caused an increase in the DNA synthesis activity of about 40% and somatostatin completely inhibited this effect being significantly effective already at the concentration of 1nM and reaching the maximal inhibition at 10nM. No further inhibition was observed for higher concentrations (100nM and 1μM).
In the rat C6 glioma cells, a different pattern of response was observed according to the intensity of the proliferating stimuli administered. In particular, the DNA synthesis induced by 10ng/ml of bFGF were completely reverted by 10 nM somatostatin, while the maximal inhibition of bFGF 50 ng/ml was observed at the concentration of 1μM. Conversely, in FCS-stimulated conditions somatostatin was completely ineffective at all the concentrations tested.
Interestingly, in the other three lines studied (U373 and DB TRG) that also express sstr subtypes able to inhibit cell proliferation (for example sstr2), somatostatin or its analogs were completely ineffective in controlling either basal or bFGF-stimulated cell proliferation. Thus, we hypothesized that the expression of specific sstr cannot represent the only requirement for the antiproliferative activity of somatostatin.
Thus, we begun to investigate for possible intracellular effectors of such an activity, that may be differently expressed in the different cell lines. Indeed, we previously reported that most of the antiproliferative effects of somatostatin, in various experimental models (for example primary cultures of human pituitary adenoma cells) are associated with an induction of a plasma membrane PTP activity. Therefore, this study has been developed looking for specific tyrosine phosphatases (PTP) as mediators of the antiproliferative activity of

somatostatin. Previously we demonstrated that PTPη, that is an almost ubiquitous PTP, may represent a crucial effector able to mediate the somatostatin control of cell proliferation.(1)

These data prompted us to investigate the possibility that the expression of PTPη may discriminate, in the different glioma cell lines, the responsivity to somatostatin. Thus by means of RT-PCR analysis we tried to establish a possible correlation between the expression and activity of this PTP and the antiproliferative effects of somatostatin. PTPη mRNA was detected in both C6 and U87MG cells while it was not detected in DBTRG and U373 cells. Interestingly, both the cell lines expressing PTPη were sensitive to the somatostatin inhibition of cell proliferation, while in the non-expressing cells somatostatin failed to control of cell growth. To established a more direct involvement of PTPη in the intracellular signalling of somatostatin we evaluated the effects of the PTP inhibitor vanadate. This treatment completely reverted the antiproliferative activity of somatostatin confirming that the activation of PTP is necessary for the somatostatin effects and suggesting a role for PTPη in such activity. Finally to better analyse the possible role of somatostatin as antitumor agent we evaluated the effect of the daily treatment with somatostatin on C6 cells proliferation after subcutaneous inoculation in nude mice. In control animals C6 cells caused the formation of fast growing tumors whose proliferation was almost completely blocked by somatostatin treatment. In conclusion from these studies we conclude that somatostatin is able to control glioma cell proliferation in vitro and in vivo through the activation of PTP and that the expression of specific PTPs in the tumoral cells represent a necessary condition for the somatostatin antiproliferative effects.

References

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