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# Indirect Antiproliferative Effect of the Somatostatin Analog Octreotide on MIA PaCa-2 Human Pancreatic Carcinoma in Nude Mice

Gisbert Weckbecker,[a] Friedrich Raulf, David Bodmer and Christian Bruns

*Preclinical Research, Novartis Pharma Inc., Basel, Switzerland*

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Analogs of somatostatin (SRIF) such as octreotide exert antiproliferative effects that are mediated directly by tumoral SRIF receptors or indirectly by down-modulation of factors that stimulate tumor growth. Direct and indirect antiproliferative effects have been demonstrated in certain SRIF receptor-positive and -negative human breast cancer models in nude mice, respectively. These antiproliferative mechanisms are also being explored in other cancer types including pancreatic cancer. While clinical pilot studies have indicated that a fraction of pancreatic adenocarcinomas respond to high-dose octreotide treatment, it is known from receptor autoradiographic and scintigraphic studies that human pancreatic carcinomas fail to express SRIF receptors, in contrast to rat pancreatic carcinomas or human endocrine pancreatic cancer. Studies on the potential anticancer effect of octreotide on the growth of experimental human pancreatic cancer and its SRIF receptor status have been controversial. Therefore, we investigated *in vivo* the effects of octreotide on the growth of MIA PaCa-2 human pancreatic carcinomas raised from cultured cells with a low passage number after receipt from the American Type Culture Collection. Nude mice bearing MIA PaCa-2 tumors were treated with a single injection of the recently developed octreotide long-acting release formulation, "SMS pa LAR." This treatment was well tolerated and resulted in a highly significant inhibition of tumor growth during weeks three and eight after administration. MIA PaCa-2 tumors were removed after eight weeks and processed for RT-PCR analysis using probes specific for each of the five somatostatin receptor subtypes $sst_1$-$sst_5$. This analysis revealed that MIA PaCa-2 tumors, like human pancreatic adenocarcinomas, do not express any of the five SRIF receptor subtypes, suggesting an indirect mode of tumor growth inhibition. In summary, the depot formulation SMS pa LAR exerted long-lasting antiproliferative effects in SRIF receptor-negative human pancreatic carcinomas in nude mice.

## INTRODUCTION

The concept of direct and indirect cell growth inihibition by somatostatin (SRIF)[b] and peptide analogs such as octreotide is used to explain their antiproliferative action in SRIF receptor-positive and negative tumors [1, 2] (Figure 1). The direct effect implies that octreotide binds to SRIF receptors on tumor cells leading to activation of a phosphotyrosine phosphatase (PTPase) and abrogation of cell growth that is induced via growth factor-regulated tyrosine kinases (TK) (Figure 1). Buscail et al. [3] demonstrated a positive correlation between the activation of a phosphotyrosine phosphatase and antiproliferative effects of RC-160 or octreotide in COS-7 and NIH 3T3 cells expressing $sst_2$. A very recent

[a] *To whom all correspondence should be addressed:* Dr. Gisbert Weckbecker, c/o Novartis Pharma Inc., Preclinical Research S-386.627, CH-4002 Basel, Switzerland. Tel.: 41-61-324-7936; Fax: 41-61-324-3576; E-mail: gisbert.weckbecker@gwa.sandoz.com.

[b] *Abbreviations:* SRIF, somatostatin; PTPase, phosphotyrosine phosphatase; TK, tyrosine kinase.

study by Srikant [4] in MCF-7 human breast adenocarcinoma cells revealed that treatment with antiproliferative concentrations of octreotide leads to a translocation of phosphotyrosine phosphatase 1C, a 66-kDa protein possessing two SH2 domains, from the cytosol to the cell membrane in a G-protein-dependent manner. Membrane-associated phosphotyrosine phosphatase 1C can promote association through the SH2 domains to phosphorylated EGF receptor, thereby terminating EGF receptor-induced mitogenic signalling. Thus, there is good evidence for the concept of direct antiproliferative action at the molecular level.

By contrast, support for the indirect mechanism is less clearcut, partially because it can be studied only *in vivo*. The indirect mechanism is apparently operative through downregulation of tumor growth stimuli including IGF-1, EGF or GH [5, 6] (Figure 1). An indirect effect may occur in cancer cells both with and without SRIF receptors. Based on the concept of the indirect effect SRIF analogs have been investigated as treatment for SRIF receptor-negative cancers including pancreatic cancer. However, models for the study of the indirect antitumor effects are rare. Controversial results were obtained in various laboratories with the MIA PaCa-2 human pancreatic carcinoma model. Gillespie et al. [7] could neither detect SRIF receptors nor an effect of RC-160 on the growth of MIA PaCa-2 cells *in vitro*. This suggested to us that MIA PaCa-2 tumors, provided that they were sensitive to octreotide in the nude mouse, would be a candidate model to study the indirect mechanism. By contrast, Radulovic et al. [8] demonstrated that this cell line was growth inhibited by RC-160 and also expressed binding sites for radiolabeled RC-160 (Kd 4.3 nM, 75,000 sites/cell or 1.75 pmol/mg protein). Such differences in SRIF receptor density could result from fluctuations in SRIF receptor (subtype) expression due to cell handling, type of media and sera used and differing cell passage number.

In this study, we used MIA PaCa-2 cells for generating tumor xenografts in nude mice in order to explore the potential antiproliferative effect of a recently developed depot formulation of octreotide, "SMS pa LAR." Tumor cell inoculation in the animals

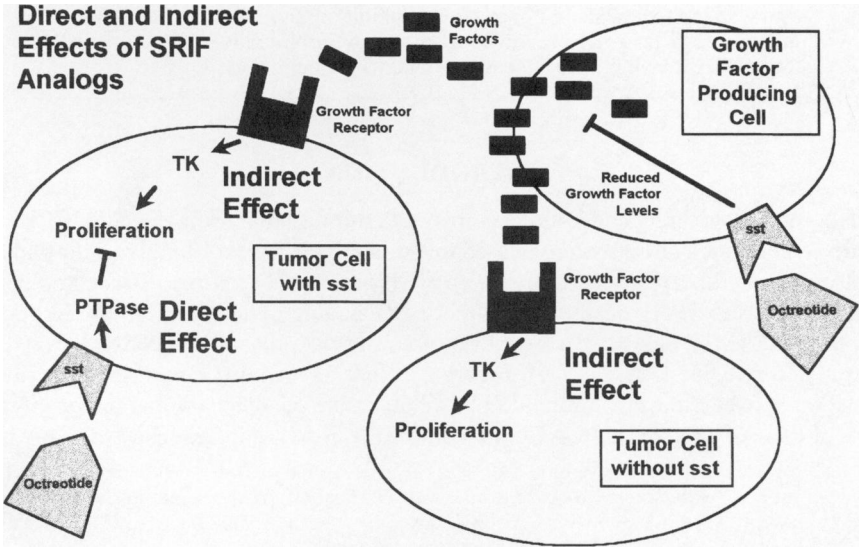


**Figure 1. Concept of the direct and indirect effects exerted by SRIF analogs such as octreotide on the proliferation of tumor cells with and without SRIF receptor (sst) expression. TK**, protein tyrosine kinase; **PTPase**, phosphotyrosine phosphatase.

was performed after a minimal number of passages following receipt of the cells from the American Type Culture Collection in order to ensure a cellular phenotype very similar to the original one.

## MATERIAL AND METHODS

The MIA PaCa-2 human pancreatic carcinoma cell line was obtained from the American Type Culture Collection (ATCC, Rockville, MD). The cells used in this study were from passage 8 to 10 after receipt from ATCC. SMS pa LAR is a depot formulation of the pamoate salt of SMS 201-995 (octreotide), which was provided by our galenical department (Dr. David Bodmer).

*Tumor growth studies in vivo*

We regularly check for the absence of mycoplasma in MIA PaCa-2 cells using bisbenzimide staining and the GenProbe hybridization assay (San Diego, CA). Cultures are propagated in DMEM supplemented with 10 percent fetal calf serum (FCS) at five percent $CO_2$. Cells are grown in the absence of antibiotics or antifungal agents. Female nude mice (nu/nu Balbc-A from Iffa Credo, Lyon, France) weighing 19-22 g, were kept in groups of five animals in macrolon cages (type III, 16 x 22 x 11 cm). The cages were placed in ventilated cabinets (Iffa Credo) that were maintained at 24 ± 1°C. The animals had free access to drinking water and a pathogen-free rodent diet (Diet A, Kliba, Basel, Switzerland). To initiate tumors from cultured cells, cells were trypsinized, and $10^7$ tumor cells (in 0.2 ml) were injected subcutaneously into both flanks of nude mice [9]. When tumors had reached a volume of approximately 0.1 $cm^3$, animals were randomized into control and treatment groups. Control animals received a subcutaneous injection of placebo, while mice in the treatment group received a single injection of SMS pa LAR, 30 mg/kg subcutaneously. Animals were continuously observed for eight weeks with weekly monitoring of tumor size and body weight. The size of the tumors was determined with a caliper. To calculate the tumor volume the equation "volume (ellipsoid) = length x depth x height x 0.52" was used. For statistical calculations, Student's t-test was applied.

*RT-PCR analysis of sst status of MIA PaCa-2 cells and tumors*

Eight weeks after injection of SMS pa LAR, tumors were removed under anesthesia (Forene® from Abbott, Cham, Switzerland) and frozen in liquid nitrogen until isolation of poly(A)$^+$-RNA for receptor subtype-specific RT-PCR analysis of $sst_1$-$sst_5$. This was performed essentially as described previously [10] except for the primer pairs for the $sst_1$ and $sst_4$ receptor specific RT-PCRs. For $sst_1$, we used the primers RS180 (5'-TCA GCT GGG ATG TTC CCC AAT G-3') and RS190 (5'-GTC GTC TTG CTC GGC GAA CAC G-3'), and for $sst_4$ the primers HS48 (5'-ACC AAC ATC TAC CTG CTC AAC CTG G-3') and HS49 (5'-GCA TAG TAG TCC AGG GGC TC-3') were used.

## RESULTS AND DISCUSSION

Nude mice bearing MIA PaCa-2 tumors were treated either with placebo or a single injection of the recently developed octreotide long-acting release formulation SMS pa LAR (Figure 2). The treatment with SMS pa LAR resulted in a highly significant ($p \leq .0002$) inhibition of tumor growth for the observation period of weeks three to eight. The mean volume of treated tumors was 25.8 percent of control at the end of the experiment. Since the body weight of octreotide-treated mice did not show the distinct drop that is usually observed with most cytotoxic anticancers, the depot formulation is apparently well tolerated (Table 1).

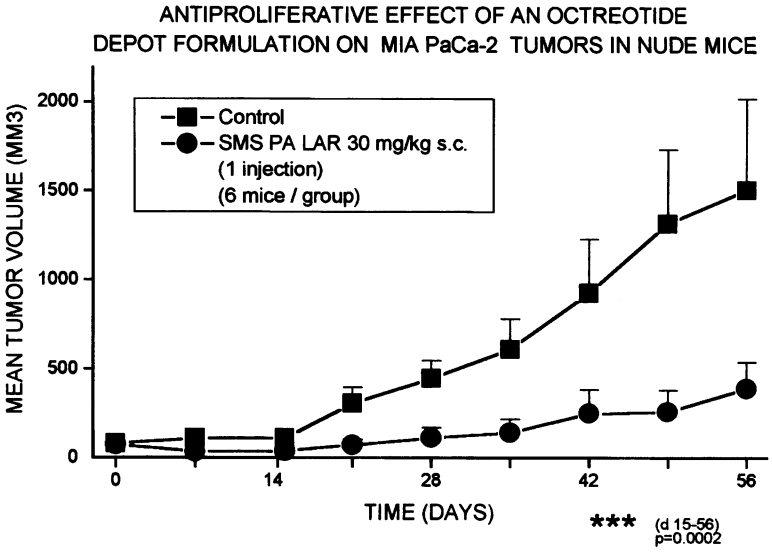


**Figure 2 Effect of a single injection of SMS pa LAR, a depot formulation of octreotide, on the growth of MIA PaCa-2 human pancreatic carcinomas in nude mice.**

The SRIF receptor status of 12 individual MIA PaCa-2 tumors was determined at the end of the eight-week experiment. Tumors were excised and processed for RT-PCR analysis using probes specific for each of the five somatostatin receptor subtypes $sst_1$-$sst_5$ (Figure 3). This analysis revealed that these MIA PaCa-2 tumors, under the experimental conditions described, failed to express detectable levels of any of the five somatostatin receptor subtypes except for two tumors that gave very weak RT-PCR signals for the $sst_3$ mRNA. It should be noted that for $sst_2$, the only human SRIF receptor with high affinity for octreotide, all tumors analyzed were negative in the sensitive human (and in addition mouse) specific RT-PCR assay. Reaction conditions were controlled by including negative and positive (human $sst_2$ cDNA) controls (Figure 3). Interestingly, there was no difference in the findings when the control tumors were compared with the SMS pa LAR-treated tumors (Figure 3). The same negative results were obtained by analyzing MIA PaCa-2 cells *in vitro* (data not shown). Thus, the MIA PaCa-2 model is apparently identical in this aspect to human pancreatic adenocarcinomas which are SRIF receptor-negative too.

**Table 1. Body weight change (g) of nude mice bearing MIA PaCa-2 tumors following injection of placebo or SMS pa LAR (same mice as in Figure 2).**

| Day | Control | ±SE | SMS pa LAR | ±SE |
|---|---|---|---|---|
| 0 | 21.9 | 0.6 | 21.1 | 0.5 |
| 7 | 22.2 | 0.4 | 21.2 | 0.4 |
| 28 | 22.3 | 0.5 | 21.4 | 0.9 |
| 56 | 23.3 | 1.0 | 22.1 | 0.9 |

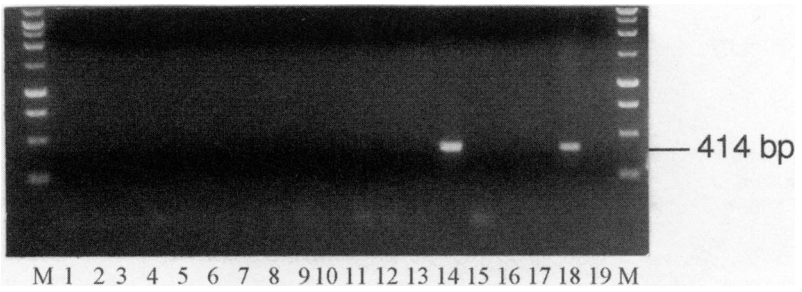


**Figure 3 RT-PCR analysis of individual MIA PaCa-2 tumors.** Gel analysis of RT-PCR products after a 40-cycle RT-PCR specific for $sst_2$. The gel shows no detectable product in the 12 individual tumor RNA preparations (lanes 1-12) and in the negative controls (lane 13 $hsst_1$, lane 15 $hsst_3$, lane 16 $hsst_4$, lane 17 hsst, cDNA, lane 19 water control). Positive controls (lane 14 human $sst_2$ cDNA, lane 18 human genomic DNA {$sst_2$ is intronless}) show the expected product of 414 bp. Tumors #1-5 were from SMS pa LAR-treated mice and single tumors #6-12 were from control animals. The amount and integrity of mRNA was verified by (β-actin RT-PCR (not shown) [10].

The precise nature of the indirect mechanism of octreotide in the MIA PaCa-2 model needs to be further addressed. Our recent studies in various species clearly indicate that octreotide leads to dose-dependent decrease of IGF-1 levels. This may be interesting in the context that pancreatic cancer growth is subject to control by the IGF-1 system. The indirect effects of octreotide on tumor growth may also include its antiangiogenic properties [11]. Reubi et al. [12] showed that veins surrounding human cancer express high levels of somatostatin receptors. Since $Tyr^3$-octreotide was bound with high affinity, the tumor-associated veins probably express $sst_2$ and/or $sst_5$. Danesi et al. [13] demonstrated the antiangiogenic properties of octreotide in various angiogenesis models *in vivo* such as the rat cornea model and the rat mesentery model.

An important factor for achieving antiproliferative effects is to maintain high octreotide plasma levels for extended time periods. In a separate experiment performed under identical conditions in the AR42J pancreatic tumor nude mouse model, the mean plasma concentration (±SE) of octreotide three weeks after a single injection of SMS pa LAR was 9.3 ± 1.6 nM. This treatment was associated with a potent inhibition of AR42J tumor growth (data not shown). Plasma levels in this range were also shown previoulsy to exert long-lasting antiproliferative effects in the ZR-75-1 mammary tumor bearing nude mouse [9].

Upp et al. [14] demonstrated that the growth of SKI human pancreatic adenocarcinomas in nude mice is inhibited by octreotide treatment. However, the SRIF receptor status of SKI tumors used in this investigation has not been reported. A very recent study by Fisher et al. [15] also showed that MIA PaCa-2 tumors in nude mice were inhibited by continuous administration of octreotide, while the other pancreatic tumor models tested were unresponsive. In contrast to our study, these MIA PaCa-2 tumors did express SRIF receptors ($sst_2$). Moreover, in a pilot clinical trial, high-dose therapy with octreotide was associated with a tumor response in a fraction of the treated patients [16]. The still limited amount of preclinical and clinical data suggest that certain pancreatic adenocarcinomas respond to SRIF analog treatment. Due to the absence of detectable SRIF receptors in human pancreatic cancer, the underlying antiproliferative effect is expected to be indirect.

In summary, a single injection of the depot formulation of SMS pa LAR was well tolerated and exerted long-lasting antiproliferative effects in SRIF receptor-negative human pancreatic carcinomas in nude mice.

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