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Failure of somatostatin to influence experimental tumor cell growth in vivo and in vitro

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Summary. The influence of somatostatin on tumor cell growth was studied in vivo in mice (sarcoma 180 ascites tumor and Lewis lung tumor) and in vitro on nontransformed and polyoma-transformed cell lines. 4 or 20 µg/100 g of cyclic somatostatin and 4 µg/100 g of linear protamin Zn-bound somatostatin were injected s.c. twice daily in the in vivo study. Cyclic somatostatin (1, 4 or 10 µg/ml) was added twice daily to the cell cultures. Somatostatin administration influenced neither the survival of animals nor the growth rate of cultured cell lines.

It has been established by many workers that somatostatin exerts an inhibitory action on various endocrine and exocrine functions[1]. In addition, it has been demonstrated that this tetradecapeptide inhibits endotoxin-induced leukocytosis and growth of granulation tissue in man and animals[2], gastrointestinal cell proliferation[3], and cell-free ribosomal protein synthesis[4,5] in rats. In a previous study we observed an inhibitory effect of somatostatin on the release of colony stimulating activity from mouse spleen lymphocytes in vitro[6]. From these results we considered the possibility that somatostatin could act as a direct inhibitor of cell proliferation. In this study we investigated the antitrophic action of somatostatin on experimental tumor cell growth in vivo and in vitro.

Material and methods. 60 male C57B1 mice, 6-8 weeks old, were injected i.p. with sarcoma 180 ascites tumor cells. 60 male DFB_1 (DBAxC57B1) mice received i.m. Lewis lung tumor cells. The animals were divided into groups of 15 (4

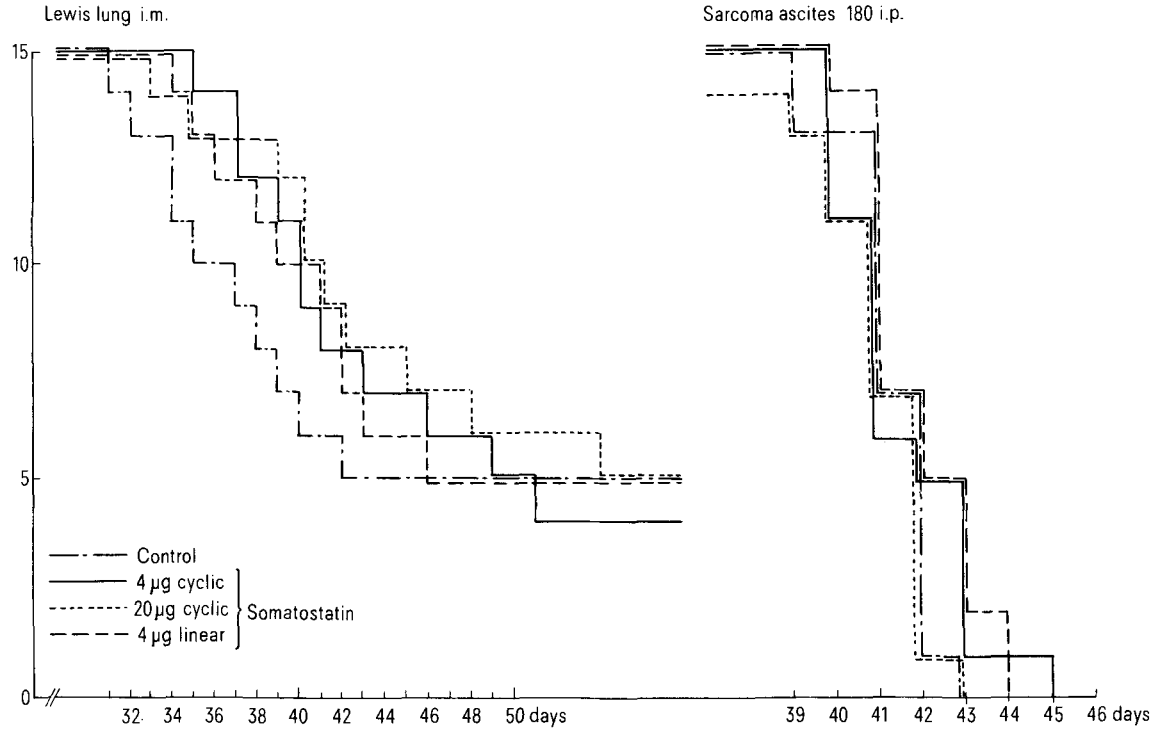

Figure 1. Effect of different doses of somatostatin on the survival of mice injected with either sarcoma 180 ascites tumor (right) or Lewis lung tumor (left). Ordinate: Number of animals alive. Abscissa: Days from tumor injection.

groups in each tumor type) and treated twice daily by s.c. injections: group A (control): saline solution; group B: cyclic somatostatin, 4 µg/100 g; group C: cyclic somatostatin, 20 µg/100 g; group D: linear somatostatin bound to protamine-Zn-sulfate, 4 µg/100 g. All somatostatin solutions were freshly prepared before injection. The treatment was started immediately after tumor cell injection. Weight was checked every 3rd day, tumor development and survival of the animals were recorded.

Cell lines of BHK-cells and polyoma-transformed and nontransformed Fisher rat cells (FR 3T3) were used for the in vitro studies. FR3T3 cells had been transformed either by polyoma wild type (=FR3T3-A2N2) or by the early temperature sensitive (ts) mutant (=FR3T3-tsaN1). FR3T3 cells and their transformants were a generous gift of Prof. F. Cuzin, Centre de Biochimie, University of Nice. Details of these cell lines are given by Seif and Cuzin[7]. Cells were grown at 37 °C and 32 °C, respectively. Medium was changed every day. Cyclic somatostatin was added to the cultures twice a day at concentrations of 1, 4, 10 µg/ml starting from the end of lag phase. Cells were daily counted in duplicates until they reached the plateau phase. Somatostatin was a product of Serono Inc., Freiburg i.Br., BRD.

Results. 1. In vivo studies. Tumor incidence was 100% in animals receiving sarcoma 180 ascites tumor cells and 80% in animals receiving Lewis lung tumor cells. No difference in tumor incidence between somatostatin-treated and control animals could be established. Similarly, the time between the tumor cell injection and the day of first detectable tumor appearance was the same in somatostatin-injected animals and in the control group. In addition, the day from which the animals started to die, the survival curves from this day on, and the total life span calculated from the day of tumor implantation, showed no statistically significant difference between the control group and any of the somatostatin-treated groups (fig. 1).

2. In vitro studies. FR3T3 cells showed a slight concentration-dependent growth inhibition at both temperatures towards the end of the growing phase (fig. 2, a and b).

BHK-cells, FR3T3 cells transformed by polyoma wild type

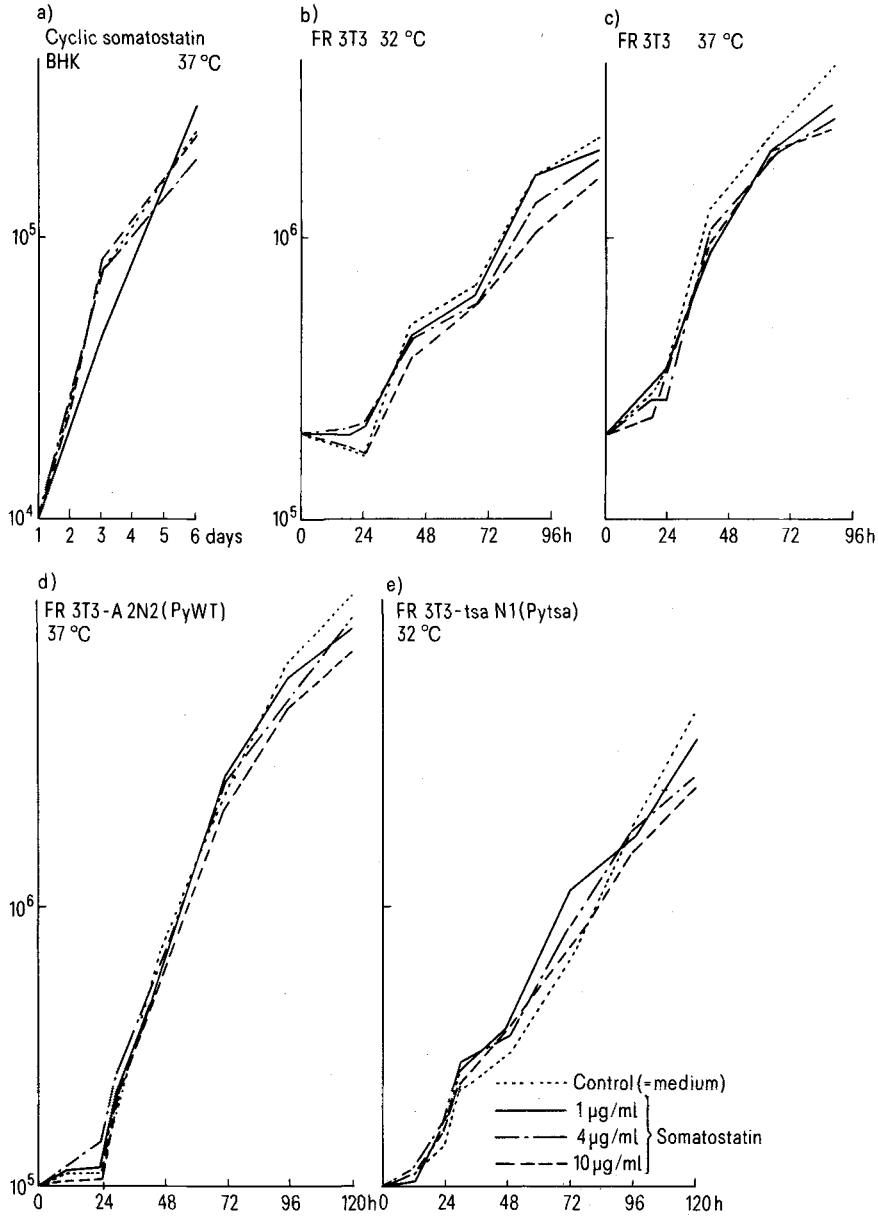

Figure 2. Cell growth in vitro without (control) and with somatostatin added to the medium in 3 different concentrations. Ordinate: Cells/plate. Abscissa: Days of incubation. a) BHK at 37 °C; b) FR3T3 (Fisher rat cells) at 32 °C; c) FR3T3 (Fisher rat cells) at 37 °C; d) FR3T3-A2N2 (transformed by Py-wild type) at 37 °C; e) FR3T3-tsaN1 (transformed by Py-ts mutant) at 32 °C.

(FR3T3-A2N2) and by the ts mutant (= FR3T3-tsaN1) are not significantly inhibited by somatostatin compared with the controls (fig. 2, c, d and e).

Discussion. Neither our in vivo nor our in vitro examinations were able to establish an inhibitory effect of somatostatin on tumor cell growth. These results are in accordance with our own experience from an unpublished pilot study in which we could not find an antitrophic effect of somatostatin, injected once daily, on various tumor types in mammals (mouse mammary tumor, Viennese leukemia, MCA-induced transplantable sarcoma, hamster melanoma, rat hepatoma, Yoshida, Lewis lung tumor, mouse myeloma, Ehrlich ascites tumor, osteosarcoma)[8].

We deliberately used very high doses of somatostatin in both the in vivo and the in vitro studies. Even when taking into account the short half-life time and the rapid metabolism of somatostatin in vivo, this high dose should give a sufficient concentration of somatostatin. So we do not believe that the failure of tumor growth inhibition is due to low dose administration. We suggest that the antitrophic effect of somatostatin reported in the literature cannot be attributed to a direct somatostatin action on growing cells but is due to an inhibition of hormones or metabolic mediators responsible for the trophic action. E.g., the inhibition of gastrointestinal cell proliferation by somatostatin is explained by inhibition of gastrin[3], the main trophic hormone of the stomach[9]; the inhibition of ribosomal protein synthesis is due to cyclic AMP inhibition by somatostatin[4,5]. We conclude from our results that there is no indication for applying somatostatin as an antitumor agent in cancer treatment.

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Argyrophilic intranuclear bodies of plant cells

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Summary. Within interphase nuclei of meristematic cells of plants there are small spherical bodies that stain intensely with silver. Their number is related to the DNA content of the species.

Silver nitrate has proved to be a useful reagent with which to stain the nuclei of plant and animal cells[1-8]. It reveals certain details of nuclear structure that are not shown up by the more commonly employed nuclear stains. In addition to the nucleolus and the nucleolar organizer region of mitotic chromosomes, both of which have a strong affinity for silver, another class of argyrophilic body is often seen within interphase nuclei of plants[5,8,9]. A nucleus may contain many of these bodies. They are small and spherical and lie in the nucleoplasm. At the ultrastructural level they probably correspond to the so-called 'dense bodies' that lie within areas of diffuse chromatin[8,10]. Since no suitable name has yet been given to them, they will be called argyrophilic intranuclear bodies (AIBs). Results are presented here on the number of AIBs within nuclei of root meristem cells of several plant species.

Materials and methods. Young root tips were removed from seedlings and were fixed and impregnated with $AgNO_3$ according to Risueño et al.[5]. The silver-impregnated apices were embedded in wax and sectioned longitudinally at a thickness ranging from 5 to 16 µm according to the species. This ensured that a high proportion of nuclei in any section were uncut. After dewaxing, the sections were mounted in Canada Balsam under a coverslip. Nuclei in the middle of the stelar portion of the meristem were selected for measurement of nuclear and nucleolar volumes and for counting the number of AIBs per nucleus.

Results. Figures 1-3 show the appearance of AIBs within nuclei of 3 species after silver staining. The diameter of the AIBs is rather variable, but the maximum diameter is about 1 µm. The largest AIBs seem to be present in species which have the fewest mean number of AIBs per nucleus (compare figs 1 and 3).

The species investigated have a wide range of nuclear DNA contents. The relationship between the mean number of AIBs per nucleus and the 2C DNA content (fig. 4) shows that species with a 2C DNA content of 5 pg or less lack, or have very few, AIBs, while in species with 5 pg of DNA or more the mean number of AIBs per nucleus increases with increasing DNA content.

The relationship between the number of AIBs and nucleolar volume bears on the question of whether the nucleolus and AIBs have a common structural basis[9-12]. Although the mean nucleolar volume of early interphase nuclei and $\log_{10}$ 2C DNA content show a positive correlation ($p < 0.01$) in the species examined, the correlation between nucleolar volume and number of AIBs is rather weak ($p > 0.05$). Moreover, a detailed examination shows that within the root apex of any one species the mean number of AIBs per early interphase nucleus is independent of the mean nucleolar volume of the same sample of nuclei (table).

Discussion. At least 2 interpretations are possible for the origin of the AIBs in plant cell nuclei. One of these interpretations, for which there is circumstantial evidence suggested by the appearance of silver-stained early interphase nuclei[4,13], is that the AIBs are clumps of prenucleolar material that have persisted into interphase[11,12]. The prenucleolar material originates from a pellicle of ribonucleoprotein (RNP) that surrounds the mitotic chromosomes[13]; the AIBs of interphase nuclei may correspond to those portions of this pellicle which did not coalesce to re-form the nucleolus at the end of telophase. The numerical interrelationships between AIBs and DNA content, and also nucleolar volume, seem consistent with this proposed origin and composition of the AIBs, since the more abundant the nucleolar and prenucleolar material, the more numerous