Tumor-specific inhibition of DNA synthesis and cell proliferation by a factor from bovine placenta – possible mechanism

Karl Letnansky

Institute for Applied and Experimental Oncology, University of Vienna, Borschkegasse 8a, A-1090 Vienna, Austria

Received 2 April 1984

A polypeptide isolated from the maternal part of bovine placentas inhibits significantly the incorporation of thymidine into the DNA of tumor cells. When normal cells are used, this effect is found only to a very limited degree. Surface membrane components have been identified which are enriched on tumor cells and which are responsible for a better binding of the inhibitor to tumor cells than to normal cells. After internalization of the receptor–inhibitor complex, a decrease in the nuclear content of two proteins is observed, which might be a requirement for the inhibition of the DNA synthesis.

DNA synthesis *Inhibitor* *Tumor cell* *Surface receptor* *Glycoprotein* *Protein synthesis*

1. INTRODUCTION

It has been reported that a factor can be isolated from bovine maternal placenta which inhibits the incorporation of thymidine into the DNA of different tumors to 43–85%, but has no significant effect on DNA synthesis of normal cells [1,2]. The development of tumors after the inoculation of cells having been incubated with the inhibitor is severely impaired, demonstrating the toxicity of this component on these cells [3]. The action of similar preparations of the same origin on tumor induction with 3-methylcholanthrene has also been described [4].

Investigations into the nature of the inhibitor demonstrated that it was inactivated by proteolytic enzymes [1] and had an apparent M_r of about 60000. Inhibitory activity is also associated with a polypeptide of roughly 110 kDa, which possibly represents a dimeric form.

Inhibition of thymidine incorporation is observed exclusively in experimental systems using intact cells and not in cell-free preparations [2]. This led us to the conclusion that the interaction of the inhibitor with surface components and the subsequent internalization of the receptor–inhibitor complex might be involved in the specific action on tumor cells. Actually, binding experiments with the inhibitor and with membranes obtained from normal and tumor cells, respectively, revealed not only the higher binding capacity of tumor membranes, but also the existence of additional receptor classes in these membranes as compared to the membranes from normal cells [2].

Experiments are here described which further document the different response of tumor and normal cells to the inhibitor. Information concerning the receptor sites is now available as well as an analysis of nuclear protein components. The latter indicates a possible influence of the inhibitor on the nuclear content of specific proteins, which might be necessary for DNA synthesis.

2. MATERIALS AND METHODS

The inhibitor was isolated and purified by gel filtration (Sephadex G-100), ion-exchange chromatography (Dowex 50W × 8, eluted with 0.15 M NaCl and, after elution of the first peak, with a gradient of 0.15 M NaCl/0.2 N NH_3) and poly-

Published by Elsevier Science Publishers B.V.
00145793/84/$3.00

acrylamide gel electrophoresis from the maternal part of fresh bovine placenta or from commerciably available, pharmaceutically used lyophilized material (Vitorgan, Stuttgart) as in [2]. Plasma membranes were prepared and purified over discontinuous sucrose gradients as in [5] from various organs and tumors of the rat. Extraction of nuclear proteins and separation on 10% polyacrylamide urea gels or 10% SDS gels have also been described [6–8]. Proteins were stained using standard procedures with amido black or Coomassie blue; carbohydrate residues (on membrane components) with the periodic acid-Schiff reagent [9]. Membrane preparations were separated employing the same procedures used for nuclear proteins. After electrophoresis, cylindrical urea gels were sliced into 1-mm thick slices, which were fixed overnight with 500 μl of 40% methanol–7% acetic acid. After standing for 2 h with 1 ml of 10 mM Tris–HCl (pH 7.4), 10 mM KCl, 1.5 mM $MgCl_2$ (which was replaced by fresh buffer after 1 h), slices were incubated for 2 h at room temperature with the inhibitor, which had been labeled by reaction with *N*-succinimidyl-[2,3-^{3}H]propionate [2]. Two hundred μl with an activity of 1300 cpm were added to each slice. After incubation, the slices were washed twice, as had been done after the fixation process, and were solubilized overnight at 54°C with 200 μl of 30% hydrogen peroxide. Samples were counted under Bray's solution in a Packard liquid scintillation spectrometer.

3. RESULTS

Significant differences in the distribution of individual surface membrane components can be detected in membranes isolated from liver cells compared to those isolated from diethylnitrosamine-induced hepatoma (not shown) or Yoshida ascites sarcoma. As shown in fig.1, this applies not only to the amount but also to the chemical nature of some of the components.

For a comparison of molecular sizes, histones isolated from rat liver nuclei were run under the same conditions. The heavy band at fraction nos 38–40 corresponds to H1 histones, while the very faint band at fractions 52 and 53 corresponds to histone H4. The remaining core histones are found in fractions 47 and 48. Small amounts of non-

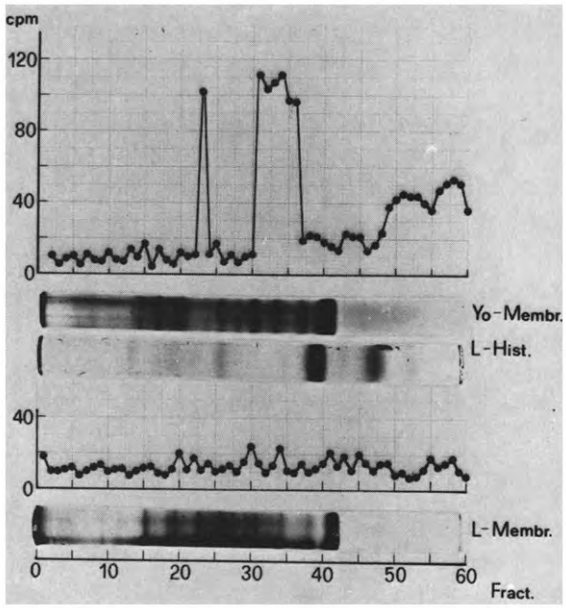

Fig.1. Binding of ^{3}H-labeled inhibitor to individual membrane fractions from Yoshida tumor cells (Yo-Membr.) and liver cells (L-Membr.) after separation on 10% polyacrylamide urea gels. Separate gels were run for staining with amido black and for the binding studies as described in section 2. One gel with liver histones (L-Hist.), run under the same conditions, served as a standard. Direction of the run was from left to right.

histone proteins in the M_r range between approx. 50000 and 120000 are spread between fractions 13 and 26.

According to the experiment depicted in fig.1, 3 main components (fractions 31, 34, 36) are enriched in the tumor membrane preparation. These components bind the inhibitor, which has been labeled by reaction with *N*-succinimidyl-[2,3-^{3}H]-propionate, to a significant degree. By contrast, the same compounds are detected in only small amounts in membrane preparations from liver cells and bind only negligible amounts of the inhibitor.

Additional binding sites of the tumor membranes are obviously associated with the polypeptide contained in fraction 23 of the gel shown in fig.1, and with low-M_r components which do not stain with amido black, but give intense reactions with the Schiff reagent. As demonstrated by the separation of liver membranes on SDS-gels (fig.2), these carbohydrate-rich fractions are well below

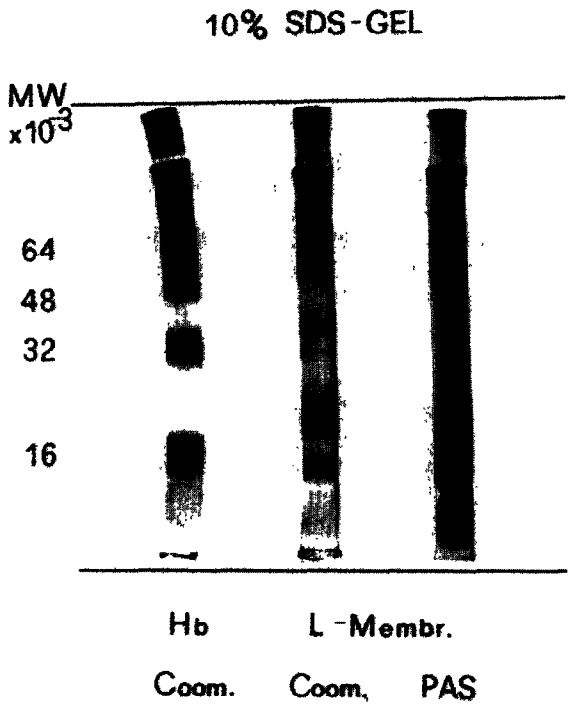

Fig.2. Electrophoretic pattern of liver plasma membranes after staining with Coomassie blue and periodic acid-Schiff reagent. Cross-linked hemoglobin served as an M_r marker.

16 kDa. In the range of compounds with high protein content, however, only small amounts of carbohydrates are found to be bound to the protein.

Further experiments were performed to obtain a better understanding of the mechanism of the inhibition. Since previous experiments have shown that the inhibitor also acts on protein synthesis, the patterns of acid-soluble nuclear proteins of Yoshida cells incubated for 1 h in the presence or absence of the inhibitor were compared. Fig.3 shows not only the histones isolated from the cells after the incubation step by a standard acid extraction procedure (the 3 heavy bands in the lower half of the gel), but also a series of non-histone proteins extracted concomitantly. Among these proteins, which have been identified only poorly until now, two bands can be located easily in the control gel, which are almost completely absent after the incubation with the inhibitor (arrows). Although there has been no experimental evidence to show a dependence of the inhibitory action of the isolated

Fig.3. Electrophoretic pattern of proteins extracted from Yoshida tumor cell nuclei [10] after the incubation of the cells for 1 h in the absence (control C) or in the presence of the decidua inhibitory factor (DIF). Arrows indicate the position of polypeptides disappearing during the incubation procedure.

factor on this decrease in certain proteins, recent results [11–14] might be an indication that one of these components participates in the assembly of the DNA polymerase–DNA complex.

4. DISCUSSION

This paper describes experiments performed to attempt an explanation of the inhibitory action of a placental component which specifically inhibits DNA synthesis in tumor cells. As far as can be said at this point, two different processes are involved in this effect: (i) the inhibitor is bound to a higher degree to the surfaces of tumor cells than to those of normal cells, which obviously results in the internalization of higher amounts of the receptor–inhibitor complex; (ii) the possible primary action within the cell is a depletion in one (or two) peptide(s). These peptides must be very labile and obviously are not replaced to a sufficient degree after the inhibition of protein synthesis. Their electrophoretic mobility is very close to that of similar components, identified in several tissues

by authors in [11–14]. These authors inhibited protein synthesis with cycloheximide and also found a significant reduction in the cellular content of a protein which had an M_r of about 53000 as well as a depression of DNA synthesis. They concluded that the multienzyme complexes required for DNA replication cannot form in the absence of this factor.

REFERENCES

[1] Letnansky, K. (1977) Österr. Z. Onkol. 4, 42–46.
[2] Letnansky, K. (1982) Biosci. Rep. 2, 39–45.
[3] Letnansky, K. (1980) in: Biomimetik als Chance (Porcher, H. and Theurer, K. eds) pp.103–109, Enke, Stuttgart.
[4] Wrba, H. (1974) Österr. Ärzteztg 29, 1351–1353.
[5] Forte, J.G., Forte, T.M. and Heinz, E. (1973) Biochim. Biophys. Acta 298, 827–841.
[6] Panyim, S. and Chalkley, R. (1969) Arch. Biochem. Biophys. 130, 337.
[7] Panyim, S. and Chalkley, R. (1969) Biochemistry 8, 3972–3979.
[8] Weber, K. and Osborn, M. (1969) J. Biol. Chem. 244, 4406–4412.
[9] Glossmann, H. and Neville, D.M. jr (1971) J. Biol. Chem. 246, 6339–6346.
[10] Tidwell, T., Allfrey, V.G. and Mirsky, A.E. (1968) J. Biol. Chem. 243, 707–715.
[11] Pardee, A.B., Dubrow, R., Hamlin, J.L. and Kletzien, R.F. (1978) Annu. Rev. Biochem. 47, 715–750.
[12] Medrano, E.E. and Pardee, A.B. (1980) Proc. Natl. Acad. Sci. USA 77, 4123–4126.
[13] Campisi, J., Medrano, E.E., Morreo, G. and Pardee, A.B. (1982) Proc. Natl. Acad. Sci. USA 79, 436–440.
[14] Lau, C.C. and Pardee, A.B. (1982) Proc. Natl. Acad. Sci. USA 79, 2942–2946.