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The mitogenic and collagen biosynthesis stimulating activities in the serum of rats with the methylcholanthrene induced sarcoma

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It is well known that the presence of serum in culture medium stimulates many physiological functions of cells grown *in vitro* [1]. The insulin-like growth factor I (IGF-I[1]), contained in the serum, is the main stimulator of cell division and collagen biosynthesis in fibroblasts and chondrocytes cultures [2 - 4]. It is produced by liver and circulates in plasma [5]. Almost the total amount of IGF-I in plasma of normal subjects exists in a form bound to high molecular weight (150 000) binding proteins (HMW-BPs). Slight amounts of IGF-I may be bound to low molecular weight (40 000 - 50 000) binding proteins (LMW-BPs). The HMW-BPs bind IGF-I and keep it in an active form, whereas the LMW-BPs bind and inactivate the IGF-I [6]. It has been demonstrated that LMW-BPs inhibit both cell division and collagen biosynthesis in chondrocytes and fibroblasts cultures [7].

Malignant disease may be associated with inappropriate IGF-I activity, manifested as mitogenic or metabolic effects. In the present study we have evaluated the mitogenic and collagen biosynthesis stimulating activities of serum from rats with methylcholanthrene-induced fibrosarcoma in cultured human skin fibroblasts.

Animals. The experiments were performed on male Wistar rats (about 200 g body weight). Tumours (fibrosarcoma) were induced by 20-methylcholanthrene (Sigma) as previously described [8]. The animals were killed by decapitation, blood was collected and submitted to clotting. The serum was collected and taken for further investigations.

Fibroblasts cultures. Human skin fibroblasts were grown to confluence in 24-well tissue culture plates as described in a previous paper [4].

The assay of mitogenic activity. The growth medium was removed, the cell layers were rinsed twice with serum free medium, and 0.5 ml of Eagle's Minimum Essential Medium without (control) or with the serum samples (5%) to be tested was added. [^{3}H]Thymidine (0.2 μCi) was added, and the cultures were incubated for 24 h at 36.6°C. Incorporation of radioactive thymidine into DNA was measured as described previously [4].

The assay of collagen biosynthesis. Collagen biosynthesis was evaluated according to Peterkofsky *et al.* [9]. The results were expressed in d.p.m. of [5-^{3}H]proline incorporated into the protein susceptible to the action of highly purified bacterial collagenase (type VII, Sigma) per μg of DNA.

IGF-I binding protein assay. The sera were treated with acetic acid at final concentration of 1 M. The endogenous IGF-I dissociated from HMW-BPs in acidic medium and was separated by gel filtration as described by Moses *et al.* [10]. Exogenous IGF-I (Imcera Bioproducts Inc.) was subjected to iodination with ^{125}I as described by Zapf *et al.* [11]. The radioactive IGF-I was incubated with the rat serum (free of endogenous IGF-I) at pH 7.6 [10]. The non-bound ^{125}I-IGF-I was separated from its protein-bound form by adsorption on charcoal

[1]Abbreviations: HMW-BPs, high molecular weight binding proteins; IGF-I, insuline-like growth factor I; LMW-BPs, low molecular weight binding proteins

and centrifugation. The supernatant, containing the protein-bound IGF-I, was subjected to gel filtration on Sephacryl S-200 column (0.9 × 63 cm) which was equilibrated with 0.1 M ammonium bicarbonate, pH 8.0 at 4°C and eluted with the same solution. Fractions of 2 ml were collected and counted in a Mini-gamma counter.

Preliminary experiments have shown that control rat serum exerts the maximal mitogenic effect on cultured fibroblasts at a concentration of 5%. Higher amounts of the serum did not increase the mitogenic response. The serum from tumour bearing rats demonstrated twice as high ability to stimulate [^{3}H]thymidine incorporation into DNA as control rat serum (Fig. 1).

The collagen-biosynthesis stimulating activities of control and tumour-bearing rat sera were also compared (Fig. 2). The incorporation of [5-^{3}H]proline into collagen of cultured fibroblasts was 6 times higher in the presence of tumour-bearing rat sera than in controls.

One can conclude that "neoplastic" serum contains a growth promoting and collagen biosynthesis stimulating factor(s). It is quite possible that this factor is IGF-I, which is known as a main serum component stimulat-

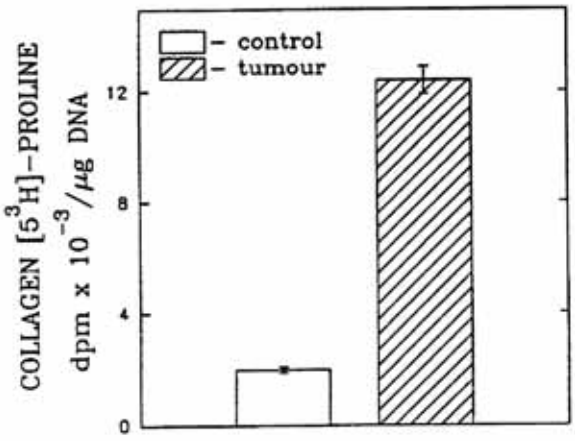

Fig. 2. Incorporation of [5-^{3}H]proline into collagen in the presence of sera from control and tumour bearing rats.
Mean values from 5 assays ± S.D.

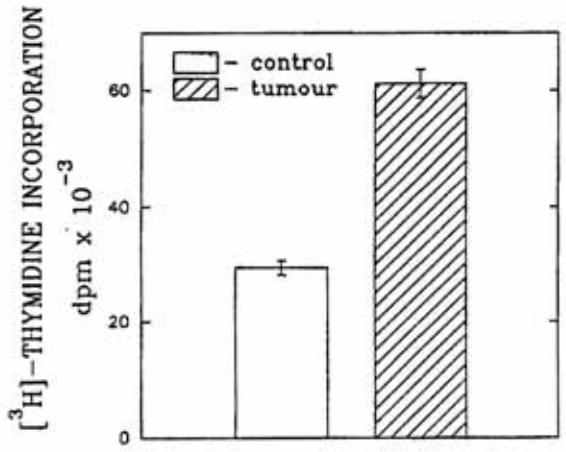

Fig. 1. Stimulation of the incorporation of [^{3}H]thymidine into DNA by sera from control and tumour bearing rats.
Mean values from 5 assays ± S.D.

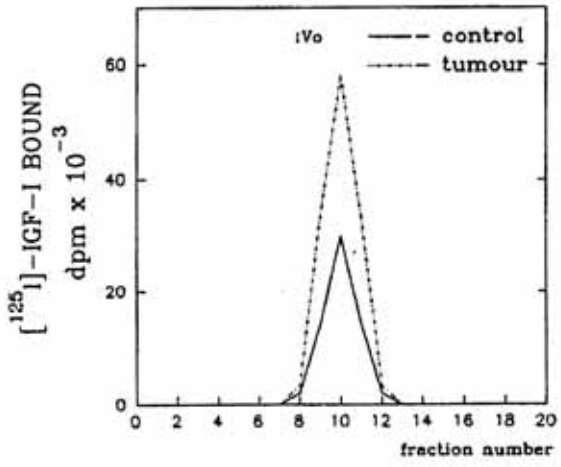

Fig. 3. Gel filtration of protein bound IGF-I on Sephacryl S-200

ing cell divisions and collagen biosynthesis in fibroblast cultures [12]. As can be seen from Fig. 3, the HMW-BPs of the tumour-bearing rats bind much more IGF-I than those of control rat serum. It is known that the amount of exogenous IGF-I bound to HMW-BPs corresponds to the quantity of endogenous IGF-I which has dissociated from the complex during the treatment of serum with acetic acid [4]. For this reason this procedure makes possible an indirect measurement of endogenous IGF-I concentration in the serum. It may be concluded that the serum of tumour-bearing rats contains more IGF-I than the respective control serum.

It is known that growth of some tumours may be enhanced *in vivo* by insulin-like growth factors [13]. Furthermore, such factors may play a role in neoplastic transformation and metastasis [13]. Immunoreactive IGF-I was detected in many tumours and, at high concentration, in the sera of tumour-bearing animals. On the other hand, the IGF-I level was not increased in the serum of patients with the lung cancer. Thus, IGF-I cannot be considered a marker of neoplastic disease [14].

We have shown previously that the rat fibrosarcoma induced by 20-methylcholanthrene is a collagen-rich tumour. The amount of collagen increases progressively during the tumour development [8]. This effect is evoked probably by IGF-I which is known to be a potent mitogenic factor and stimulator of collagen biosynthesis.

REFERENCES

1. Ellem, K.A.O., Gierthy, J.F. (1977) *J. Cell. Physiol.* **92**, 381 - 400.
2. Fine, A., Poliks, Ch.F., Donahue, L.P., Smith, B.D., Goldstein, R.M. (1989) *J. Biol. Chem.* **246**, 16991 - 16998.
3. Goldstein, R.M., Poliks, Ch.F., Pilch, P.F., Smith, B.D., Fine, A. (1989) *Endocrinology* **124**, 964 - 970.
4. Pałka, J., Bird, T.A., Oyamada, I., Peterkofsky, B. (1989) *Growth Factors* **1**, 147 - 156.
5. Scott, C.D., Martin, J.L., Baxter, R.C. (1985) *Endocrinology* **116**, 1094 - 1099.
6. Hardouin, S., Hossenlopp, P., Segovia, B., Seurin, D., Portolan, G., Lassarre, C., Binoux, M. (1987) *Eur. J. Biochem.* **170**, 121 - 132.
7. Peterkofsky, B., Pałka, J., Wilson, S., Takeda, K., Shah, V. (1991) *Endocrinology* **128**, 1769 - 1779.
8. Sobolewski, K., Bańkowski, E. (1984) *Acta Biochim. Polon.* **31**, 317 - 328.
9. Peterkofsky, B., Chojkier, M., Bateman, J. (1982) in *Immunochemistry of the Extracellular Matrix* (Furthmayer, M., ed.) vol. 2, pp. 19 - 47, CRC Press, Boca Raton FL.
10. Moses, A.C., Nissley, S.P., Short, P.A., Rechler, M.M., White, R.M., Knight, A.B., Higa, O.Z. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 3649 - 3656.
11. Zapf, J., Walter, H., Froesch, E.R., (1981) *J. Clin. Invest.* **68**, 1321 - 1330.
12. Macaulay, V.M. (1992) *Br. J. Cancer* **65**, 311 - 320.
13. Kaleko, M., Rutter, W.J., Miller, A.D. (1990) *Mol. Cell. Biol.* **10**, 464 - 476.
14. Macaulay, V.M., Teale, J.D., Everard, M.J., Joshi, G.P., Millar, J.L., Smith, L.E. (1988) *Eur. J. Cancer Clin. Oncol.* **24**, 1241 - 1249.