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Growth Self-Incitement in Murine Melanoma B16: A Phenomenological Model

Abstract. *The growing murine melanoma B16 secretes increasing quantities of a substance or substances immunologically cross-reactive with insulin. The elevated concentrations of these substances in blood are accompanied by a decrease in blood glucose concentration and release of growth hormone, which is followed by increased tumor growth. By use of a phenomenological model based on these data, we show that B16 incites its own growth by positive feedback.*

Certain human (*1–5*) and murine (*6–8*) tumors produce and secrete a substance or substances immunologically cross-reactive with insulin (SICRI's). Several features distinguish SICRI's from insulin and show that they are of tumor origin: (i) their high concentrations observed also in tumor-bearing diabetic patients (*2, 5*) and diabetic mice (*6–9*); (ii) the restoration of normal insulin concentrations after removal of the tumor (*1, 2, 4*); (iii) the high concentrations of SICRI's within tumor tissue (*1, 2, 9, 10*); and (iv) the lack of a correlation between concentrations of circulating SICRI's and C-peptide (*5, 11*). Yet SICRI's display insulin-like action in that they decrease blood glucose in tumor patients (*2–5*) and tumorous mice (*6, 8, 9*).

We now show that in murine melanoma B16 the concentration of SICRI's in blood is a function of tumor volume and that glucose concentration in blood is a function of SICRI concentration. The decreased amount of glucose in blood is correlated with elevated amounts of circulating growth hormone which, in turn, is paralleled by increased tumor growth. By use of a phenomenological model based only on correlations of tumor volumes and SICRI and glucose concentrations in blood, we show that a positive feedback—that is, growth self-incitement—occurs in melanoma B16.

Male C57BL/H Irb mice 2.5 months of age and weighing 22 g each were housed five to a cage and given free access to water and standard pelleted food. The tumor, originally obtained from the Holt Radium Institute (Manchester, England), has been maintained at the Rugjer Bošković Institute since 1975 by subcutaneous inoculations of 2×10^6 cells into the flanks of recipient animals. Three opposite diameters (A, B, and C) of almost spherical prolate ellipsoid tumors were measured, and their volume was calculated as $V = ABC\pi/6$. Blood glucose concentrations were measured by the ortho-toluidine method (*12*). The SICRI concentrations were determined by insulin-specific radioimmunoassay (*13*) with the use of Phadebas kits (Uppsala, Sweden); therefore, these concentrations are relative and expressed as insulin equivalents (*5*).

Secreting tumors release SICRI's even in alloxan-diabetic mice (*6*). In normoinsulinemic animals with melanoma B16, SICRI concentrations in the blood may be more than five times greater than normal insulin concentrations and are correlated with tumor volume (Fig. 1A); SICRI's also appear in diabetic melanoma-bearing mice (*9*). By fractionating tumor extract on a Sepharose 6B column, we obtained an apparent relative molecular size for B16 SICRI of 120,000 (*10*), as in non-Hodgkin's lymphoma (*5*). Increase of tumor volume and of SICRI concentrations was accompanied by a decrease in blood glucose concentration (Fig. 1B). The correlation between amounts of SICRI and blood glucose was high.

Our phenomenological model is based on consideration of (i) exponential volume-SICRI and SICRI-glucose relations (Fig. 1) and (ii) the Gompertzian tumor growth model (*14*) modified to include positive feedback and chosen empirically because of its demonstrated applicability to tumor growth (*14*). Figure 2 shows the proposed feedback loop formulated by the following relations.

$$S = ae^{\alpha V};\ a,\alpha > 0 \tag{1}$$

$$G = be^{-\beta S} = be^{-a\beta e^{\alpha V}} = g(V);\ b,\beta > 0 \tag{2}$$

$$V = f(G,t) = V_0\, e^{P_n(G)(1\ -\ e^{-\gamma t})};\ V_0, \gamma > 0 \tag{3}$$

$$P_n(G) = a_1 + a_2G + \ldots + a_nG^{n\ -\ 1} \tag{4}$$

where S and G denote SICRI and glucose concentrations, respectively, V_0 the initial tumor volume, and V the tumor volume at time (t) after transplantation of the tumor. The symbols a, b, α, β, γ, a_1, a_2, . . . , a_n are parameters obtained by the least-square fitting of the empirically chosen functions 1 to 3 to the data (see Table 1). The parameter a_2 differs significantly from zero, while a_3 (and also a_4, a_5, . . . , a_n) can with fair confidence be taken as zero according to the value of F [see (*15*)]. Thus, volume appears to depend significantly on glucose concentration; this dependence is described by a simple exponential function.

The feedback can be measured by calculating the open-loop gain parameter (Ω) [see (*16*)]. Here the infinitesimal changes of tumor volume (dV) and glucose concentration (dG) according to Eqs. 2 and 3 are

$$dV = \left.\frac{\partial f(x,\ t)}{\partial x}\right|_{x\ =\ G} dG + \left.\frac{\partial f(G,y)}{\partial y}\right|_{y\ =\ t} dt \tag{5}$$

$$dG = \left.\frac{\partial g(z)}{\partial z}\right|_{z\ =\ V} dV \tag{6}$$

Table 1. Parameters obtained by fitting the function $\ln V = \ln V_0 + P_n(G)(1 - e^{-\gamma t})$ to the 85 data sets for tumor volume (V), glucose concentration in blood (G), and time (t). For a given parameter γ, the multiple linear regression analysis was performed. By varying γ, the absolute minimum of χ^2 was obtained. The goodness of the entire fit (F_R) was tested by the F test for the multiple correlation coefficient R, and the inclusion of each additional term was tested by the F test for χ^2 (F_χ) [see (*15*)]; ν is the number of degrees of freedom.

ν	γ (day^{-1})	V_0 (mm^3)	a_1	a_2 (mM^{-1})	a_3 (mM^{-2})	R	F_R	χ^2/ν	F_χ
82	3.0×10^{-4}	48 ± 10	$(5.3 \pm 0.2) \times 10^2$	0	0	0.8790	282	0.32634	
81	3.15×10^{-2}	38 ± 7	8.5 ± 0.4	−0.12 ± 0.01	0	0.9475	360	0.14862	99.1
80	3.455×10^{-2}	36 ± 6	8.3 ± 0.4	−0.16 ± 0.03	$(1.3 \pm 0.9) \times 10^{-3}$	0.9487	243	0.14725	1.8

Equations 5 and 6 can be considered as the linearization of the proposed feedback loop. With no feedback, $dG = 0$, and the change in tumor volume dv is then

$$dv = \left.\frac{\partial f(G, y)}{\partial y}\right|_{y = t} dt \qquad (7)$$

By combining Eqs. 5 through 7 we obtain

$$dv = (1 - \Omega)\, dV \qquad (8)$$

$$\Omega = \left.\frac{\partial f(x, t)}{\partial x}\right|_{x = G} \left.\frac{\partial g(z)}{\partial z}\right|_{z = V} \qquad (9)$$

In the present case, the parameter Ω as a function of V is given by

$$\Omega(V) = \alpha V g(V) \left[\ln \frac{g(v)}{b}\right] \left(\ln \frac{V}{V_0}\right) \frac{\sum_{i=1}^{n} (i - 1)\, a_i [g(V)]^{i-2}}{P_n[g(V)]} \qquad (10)$$

For melanoma B16, the parameter Ω is positive and differs significantly from zero for all tumor volumes between 55.3 mm^3 and the observed maximum 15,063 mm^3 (Fig. 3).

Standard deviations of the parameters a, b, α, β, V_0, a_1, and a_2 were obtained by the accepted regression analysis procedures, and the errors were evaluated by use of standard formalism for error propagation [see Table 1, Eq. 10, and (*15*)].

With the use of Eqs. 8 and 10, we can visualize the contribution of the feedback to the enhancement of tumor growth. By integration of Eq. 8 under the condition $v(V_0) = V_0$, we obtain a relative enhancement parameter denoted V_E (see Fig. 4).

$$V_E = \frac{V - v}{v} = \frac{\int_{V_0}^{V} \Omega(z)\, dz}{V - \int_{V_0}^{V} \Omega(z)\, dz} \qquad (11)$$

Equations 2 and 3 represent a system of nonlinear equations for V and G that characterizes the proposed feedback loop. Solutions of the system, G' and V', are functions of time and represent the operating points (*16*) that determine the

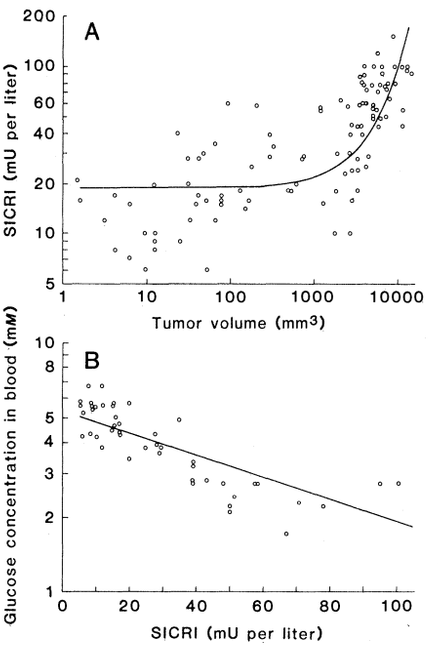

Fig. 1 (left). Regression analysis of tumor volume and concentration of a substance or substances immunologically cross-reactive with insulin (SICRI) in blood (presented as a log-log plot) (A) and of SICRI and glucose (presented as a log-lin plot) (B) in mice bearing melanoma B16. The function $\ln S = \ln a + \alpha V$ was fitted to 103 SICRI-volume data pairs (A) by the least-square fitting to the straight line, yielding $a = 19 \pm 1$ mU per liter, $\alpha = (1.6 \pm 0.1) \times 10^{-4}$ mm^{-3}, the correlation coefficient $r = 0.74$, and $\chi^2/\nu = 0.286$ (ν is the number of degrees of freedom). The same fitting procedure was applied to the 44 glucose-SICRI data pairs (B) with the function $\ln G = \ln b + \beta S$, giving $b = 5.3 \pm 0.2$ mM, $\beta = (1.0 \pm 0.1) \times 10^{-2}$ mU per liter, $r = -0.81$, and $\chi^2/\nu = 0.044$.

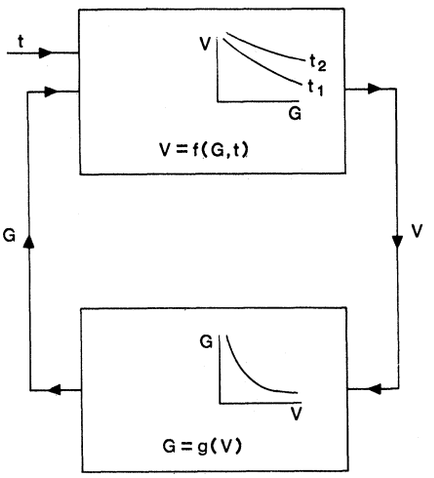

Fig. 2 (right). The feedback loop of the tumor growth self-incitement model. The symbols are described in the text.

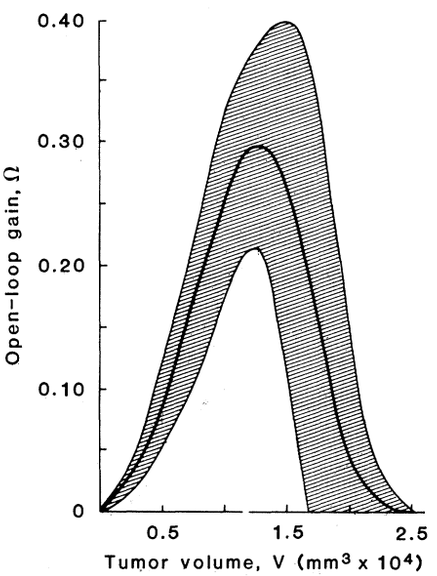

Fig. 3. The open-loop gain parameter $\Omega \pm 2$ standard deviations as a function of tumor volume.

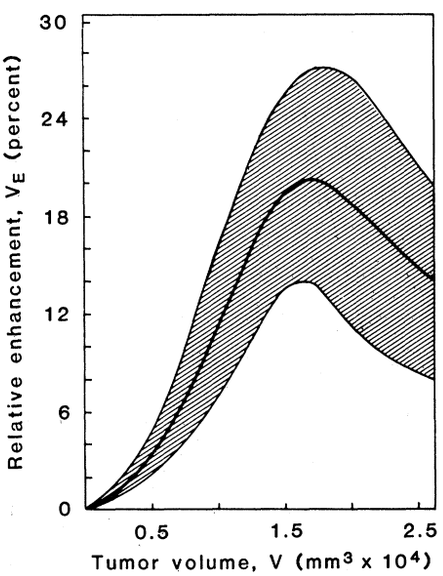

Fig. 4. The relative enhancement $V_E \pm 2$ standard deviations by the positive feedback as a function of tumor volume.

actual tumor volumes and glucose concentrations in nondiabetic mice. Thus, the parameters Ω and V_E must be calculated for volumes V'. The value of V' ranges from 38 ± 14 mm^3 ($\equiv V_0$) to 4864 ± 3254 mm^3 at day 30 (the last day of the experiment). (The estimated errors of V' are 2 standard deviations obtained by propagating the error of the parameters of the model.) For realistic values of V' in our system, self-incitement contributes up to 10 percent (Fig. 4).

Our model does not indicate mechanistic details of self-incitement; here SICRI's and glucose provide only phenomenological correlates of the tumor-proliferating activity. However, some mechanisms can be envisaged. For example, an increase in the amount of circulating growth hormone parallels tumor growth, elevations in SICRI concentration, and decreases in glucose concentration (*3*, *10*). In tumor extracts no growth hormone was found, showing that this substance, unlike SICRI's, is not of tumor origin. Moreover, growth hormone concentrations correlate well with those of blood glucose ($r = -0.59$) (*10*), and depression of blood sugar amount is known to cause the release of growth hormone into the circulation (*17*). This hormone could close the feedback loop by inciting tumor proliferation; tumor-promoting effects of growth hormone on some (mainly lymphoproliferative) tumors are well documented (*18*). Our ongoing experiments do not exclude autocrine, paracrine, or endocrine activity of SICRI's, in analogy with what has been suggested for the action of various growth factors (*19*). Fluctuations in glucose concentrations related to SICRI's have been reported in patients bearing different types of tumors (*1–5*). Furthermore, changes in the concentration of growth hormone in response to such fluctuations have been shown to occur in Hodgkin's disease (*3*).

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Heparin Binds Endothelial Cell Growth Factor, the Principal Endothelial Cell Mitogen in Bovine Brain

Abstract. *Endothelial cell growth factor (ECGF), an anionic polypeptide mitogen, binds to immobilized heparin. The interaction between the acidic polypeptide and the anionic carbohydrate suggests a mechanism that is independent of ion exchange. Monoclonal antibodies to purified bovine ECGF inhibited the biological activity of ECGF in crude preparations of bovine brain. These data indicate that ECGF is the principal mitogen for endothelial cells from bovine brain, that heparin affinity chromatography may be used to purify and concentrate ECGF, and that the affinity of ECGF for heparin may have structural and perhaps biological significance.*

Endothelial cells in vivo are responsible for the formation of a nonthrombogenic interface between blood and tissue and have a prominent mechanistic role in the pathophysiology of angiogenesis, wound repair, thrombosis, and atherogenesis (*1*). Thus the discovery of factors that regulate endothelial cell proliferation and differentiation can contribute to our knowledge of the developmental biology and physiology of the vascular system (*2*).

Endothelial cell growth factor (ECGF) is an acidic polypeptide mitogen—purified and characterized from bovine neural tissue (*3*)—which supports the proliferation and serial propagation of human endothelial cells in vitro (*4*). The addition of heparin to a crude preparation of ECGF increases the specific activity of the ECGF preparation as an endothelial cell mitogen, reduces the endothelial cell population doubling time, and permits the establishment of stable human endothelial cell clones (*5*). Although the mechanism of heparin action is unclear, the synergistic mitogenic activity between the anionic carbohydrate and acidic polypeptide mitogen (*3*) suggests the possibility of a structural interaction between these biological response modifiers. We report that (i) the anionic polypeptide ECGF is the principal endothelial cell mitogen in extracts of bovine neural tissue and (ii) heparin-Sepharose chromatography can be used to purify ECGF. We further suggest that the biological activity of heparin as a potentiator of ECGF activity may involve a structural interaction between the carbohydrate and the polypeptide.

Crude preparations of ECGF from bovine brain, at neutral *p*H and low ionic strength, were subjected to streptomycin sulfate precipitation (*6*). Fractions containing the 20K, low molecular weight ECGF were obtained from gel exclusion chromatography of the bovine brain extract (*3*). These fractions were used as the starting material for heparin-Sepharose studies; half-maximum stimulation of human endothelial cell growth occurred at approximately 5 μg/ml.

Preparations of low molecular weight ECGF were processed through a column consisting of heparin covalently coupled to Sepharose 4B. Most of the protein passed through the column and contained no human endothelial cell growth–promoting activity (Fig. 1A). Elution of the heparin-Sepharose column with 1*M* NaCl resulted in the recovery of the endothelial cell growth–promoting activity. The titration of the biological activity present in these fractions revealed that the mitogen was capable of promoting half-maximum human endothelial cell growth at concentrations of 10 to 15 ng/ml (Fig. 1B). The significant increase in the specific activity of ECGF could also