EFFECT OF EXOGENOUS RNA ISOLATED FROM BONES AND EMBRYONIC TISSUES ON GROWTH OF SARCOMA M1

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UDC 616.006.3.04:092.9:547.963.32

A preparation of total RNA isolated from bone inhibits growth of sarcoma M1 and changes its morphology. Treatment of the RNA with ribonuclease completely abolishes its action, but treatment with trypsin causes no change in this effect.

Many investigators [7, 8, 11] consider that tumor cells differ from normal cells by disturbances of the system controlling biosynthetic processes. Because of the importance of epigenome disturbances in the mechanism of carcinogenesis, several workers have investigated the action of a total preparation of RNA on growth of tumors [1-4, 12, 14, 16, 17]. These investigations showed that RNA isolated from homologous tissues reduces the rate of successful transplantation, inhibits development, and changes the morphology of the transplanted tumor. Meanwhile, RNA from the liver had no effect on cells of sarcoma 45 under the same experimental conditions, i. e., the action of RNA possessed definite specificity [1, 5]. Other experiments showed that RNA from tissues homologous with the tumor did not always have an inhibitory action (RNA from granulation tissue and embryonic tissues of rats when used to treat rat sarcoma 45 [2, 5]).

Hence, despite the interesting theoretical aspects of these experiments, the contradictions associated with them required further investigations, especially with regard to tumors of connective-tissue genesis.

The object of the investigation described below was to study the action of RNA isolated from bones and embryonic tissues on tumor growth.

EXPERIMENTAL METHOD

The animals used in the experiments were 128 noninbred rats weighing 80-90 g.

In the control series, a suspension of tumor cells of sarcoma M1 [9] was incubated in Hanks's solution at 37° for 1 h and then injected subcutaneously into rats, giving 100% of successful transplantations.

In the experimental series RNA was added to the incubation medium. RNA was isolated from bones of rats weighing 120-130 g and from rat embryos from which the internal organs, head, and skin were removed. RNA was obtained by the Kirby–Georgiev method as modified by Aksenova et al. [1]. The RNA preparations contained traces of DNA (determined by Dische's method [13]) and of protein (determined by Lowry's method [15]). RNA isolated from bone tissue was added to the incubation medium, which contained 800,000 cells, in doses of 48 and 50 μg/ml. RNA from embryonic tissues was added in doses of 50, 500, and 1000 μg/ml incubation medium, which also contained 800,000 cells. To verify the action of RNA, ribonuclease (Serva) was added to the resulting total preparation RNA in a dose of 30 μg/ml. Treatment continued for 1 h at 37°. The ribonuclease was removed by double deproteinization with phenol, the product was washed with ether until it became clear, and was heated on a water bath until the ether vapor disappeared (at 40°). The ribonuclease hydrolysate was added to the incubation medium and incubation carried out for the same period of time.

Professor M. I. Sitenko Research Institute of Prosthetics, Orthopedics, and Traumatology, Khar'kov. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 70, No. 9, pp. 72-75 September, 1970. Original article submitted September 11, 1969.

TABLE 1. Effect of RNA Preparation Isolated from Bone on Growth of Sarcoma M 1

Nature of treatment	No. of Animals	Mean weight g	P	Coefficient of inhibition, %
RNA (48 μg/ml)	5	6,8±2,3	<0,05	38,1
Control	3	11,0±1,2		
RNA (50 μg/ml)	4	6,1±1,3	<0,05	59,3
Control	4	17,0±5,1		
RNA (50 μg/ml)	10	5,5±1,0	<0,02	56,0
Control	10	11,9±3,5		
RNA (50 μg/ml)	6	12,3±2,5	>0,05	—
Control	6	17,1±2,0		
RNA after treatment with ribonuclease	6	9,8±1,8	>0,05	—
Control	6	10,1±0,2		
RNA after treatment with trypsin	7	9,1±1,0	<0,05	44,1
Control	7	16,3±2,8		

To rule out the possibility of action of traces of protein bound with the RNA, the preparation was treated with trypsin (Difco) in a dose of 100 μg/mg RNA, followed by double deproteinization, washing with ether, and precipitation with alcohol. After treatment with trypsin, the RNA was added to the incubation medium and incubation was carried out for the same period.

The state of the cells after incubation, in both the control and experimental series, was tested on the basis of their ability to accumulate granules of a 0.1% solution of neutral red.

On the 20th day the animals of the experimental and control groups were sacrificed, and the tumors were removed and weighed. Material was taken from each tumor for histological examination (staining with hematoxylin-eosin and by Van Gieson's method).

To study changes in the state of the cells under the influence of RNA isolated from bone tissue, comparative cytokaryometric examinations were made of impressions of the sarcoma M1 tumor in the control and experimental series. In each preparation 100 cells and their nuclei were measured. The numerical results were subjected to statistical analysis by the Student–Fisher method.

EXPERIMENTAL RESULTS

The experimental results are given in Table 1. They show that incubation of tumor cells with RNA from bone tissue led to a decrease in the number of successful transplantations, by between 38 and 59%, except in one series when the weight of the tumors in the experimental group was reduced by only 28%, this decrease not being significant. Treatment of the RNA preparation with ribonuclease completely abolished the inhibitory effect, but its treatment with trypsin left this effect unchanged. The action of the preparation can thus be considered to be dependent on RNA and not on the traces of protein left behind after double deproteinization during preparation of the RNA specimen.

RNA isolated from embryonic tissues had no action on growth of sarcoma M1 in doses of 50, 500, and 1000 μg, nor had it any action after treatment with ribonuclease and trypsin.

Under the influence of RNA from bone tissue, the morphological characteristics of the developing tumors were slightly modified, for whereas in the control group the tumor consisted of an undifferentiated polymorphocellular sarcoma, subdivided by ill-defined bands of collagen fibers and vessels of capillary type, containing large cells with basophilic cytoplasm, a round hyperchromic nucleus, and large granules of chromatin, in the experimental series on the 12th day the tumor consisted of a large number of small cells with a round and polymorphic nucleus, grouped in small nodules.

The results of the cytokaryometric investigation showed that the size of the cells on the 12th day in tumors developing from cells of sarcoma M1 treated with RNA was 12.3% smaller than in the control group (not treated with RNA). The area of the nucleus was also 6% smaller than in the control (in both cases $P < 0.05$).

This investigation thus showed that whereas a total preparation of exogenous RNA isolated from embryonic tissues, if incubated with tumor cells of sarcoma M1, does not modify their properties and, in particular, their subsequent growth and morphology, a total preparation of exogenous RNA isolated from bone inhibits growth of the tumor and modifies the cytological characteristics of the cells, causing a decrease in volume of both nucleus and cell.

These experiments indicate merely an inhibitory effect of the total RNA preparation; further investigations aimed at elucidating the mechanism of this action are required. The suggestion has been put forward that exogenous RNA, by its action on the genetic apparatus of the cell and by causing repression and depression of some structural genes of tumor cells, reduces the degree of their autonomy [1]. Work of particular interest has been carried out by Tomsons [10], who investigated the effect of exogenous RNA on synthesis of proteins and nucleic acids in ascites cells of Ehrlich's carcinoma. Having observed that exogenous RNA can penetrate in a polymerized state into cells and can program protein synthesis, Tomsons concludes that inhibition of synthesis of RNA and proteins under the influence of exogenous RNA may be connected with the ability of this compound to induce the synthesis of histone-like proteins which can act as inhibitors of these processes. The many different possible aspects of the mechanism of the inhibitory action of RNA require further investigation.

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