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Activation Of Anti-Oncogene P53 Produced By Embryonic Extracts In "In Vitro" Tumor Cells.

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It is clear from the literature that pregnant uteri extracts taken from C57BL/6 syngeneic mice during embryonic differentiation, such as Drosophila at blastodermic stage extracts, significantly inhibit the growth of Lewis tumor "in vivo". These results have been interpreted as a consequence of the regulation of genes involved in cell differentiation and apoptosis. In this study we examined the effects of embryonic extracts on different types of "in vitro" tumor cells: glioblastoma, hepatocarcinoma and melanoma. The activation of p53 anti-oncogene was evaluated after the administration of 100 microlitres of the following embryonic extracts: 1°) Zebrafish: a) middle blastula-gastrula, b) 5 somites, c) 20 somites; 2°) Trout: a) middle blastula-gastrula, b) 5 somites, c) 20 somites. The activation of P53 anti-oncogene was evaluated with immuno-histochemical and analytical flow cytometry technique. We recorded a significant activation of P53 anti-oncogene after treatment with embryonic extracts. However, these effects were limited to certain types of embryonic extracts. We mapped the extracts which had the most significant effect on P53 anti-oncogene activation in glioblastoma cells. This study raises the possibility of further research on regulators present in the embryo during cell differentiation and seems to open up a way for the physiological control of malignancy.

Introduction

The evidence obtained from studying the interaction between tumor cells and embryonic tissues suggests that tumor development in the embryo is reduced or supressed when processes of differentiation are in progress (1, 2). The administration of known carcinogens during cell differentiation in the embryo causes some malformations in offspring, but no tumor induction. Once organogenesis is complete, the frequency of tumor induction rises with a concomitant decrease in the rate of malformations (3, 4, 5). These findings could indicate that cancer can be viewed as a developmental deviation susceptible to control by regulators of cell differentiation. In fact, we know that cell differentiation is a specific and selective process that represses the proto-oncogenes and activates the anti-oncogenes and that gene regulation during organogenesis takes place on the basis of a network of substances which cooperate in several cascades. In the past, this

hypothesis led us to perform some experiments "in vivo". We implanted C57BL/6 syngeneic mice, together with 10^6 cells of Lewis Lung Carcinoma, one of the following extracts: 1) embryos of syngeneic mice at day 9 of pregnancy, 2) uteri of syngeneic mice at day 9 of pregnancy, 3) non pregnant uteri of syngeneic mice, 4) embryos of Drosophila at blastodermic stage, 5) normal livers of syngeneic mice (6, 7). The results of these experiments demonstrated that pregnant uteri extracts, such as Drosophila embryonic extracts at blastodermic stage, significantly inhibited the growth of Lewis tumor. No effects were observed with extracts of non pregnant uteri, mammals' embryos and normal livers. These results may indicate that regulatory genes involved in cell differentiation and apoptosis are activated by the above mentioned extracts. The regulatory substances could be present in the uterus of mammals. In this sense the uterus could represent not only a nutritional organ but a regulatory organ of differentiation as well. In addition, these regulators could be present in the embryo of ovipari. In fact, the embryo of ovipari contains in itself all the necessary information to complete differentiation and development. The aim of the present research is to determine whether it is possible to regulate the expression of p53 in diverse in vitro tumor cells by different embryonic extracts.

Materials and Methods

The embryos of Zebrafish and trout at the stages of middle-blastula-gastrula, 5 somites, 20 somites were washed in distilled water and placed in a solution of pure glycerine and 30% ethylic alcohol at the ratio 4 to 1. The embryos of Zebrafish were sonicated with 2 cycles of 10 seconds each and further treated with a turboemulsifier. The embryos of trout were processed with a turboemulsifier for 3 minutes and then were vacuum filtered through millipore 90 microns membranes and subsequently 10 microns membranes.

100 microliters of these solutions were incubated for 48 hours with the following culture of stabilized tumoral cells: 1) glioblastoma, 2) melanoma, 3) hepatocarcinoma. The cells of glioblastoma and hepatocarcinoma were grown in Ham F12 containing antibiotics and 5% heat-inactivated fetal serum (FCS), while melanoma was cultured in the same medium containing 20% FCS.

Other technical details of cell preparation for analysis have already been described (8).

The evaluation of p53 protein was effected with analytical flow cytometry technique and with immunohistochemical method using the alkaline phosphatase detection system. With this last method the intensity of red immunostaining was evaluated in a four-step scale (from 0 to +++): 0 = no activation of p53; + = low activation of p53; ++ = medium activation of p53; +++ = high activation of p53. We labeled from X1 to X6 the different extracts and we performed single- blind experiments.

Results

In fig.1 the activation of p53 in the treated cells of glioblastoma with X1 extract is compared with the untreated cells. The treated cells with analytical flow cytometry technique revealed a 23.6% higher activation.

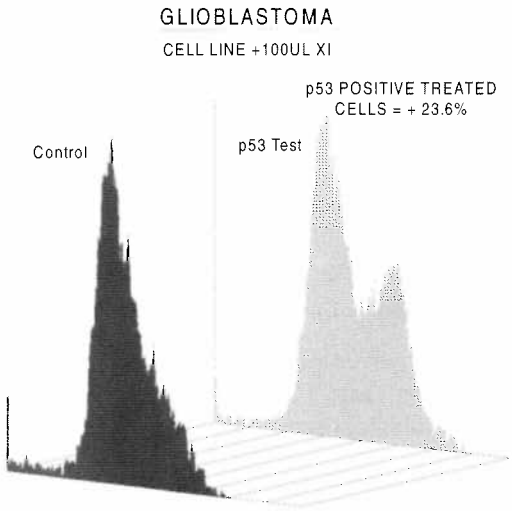

Fig. 1: Activation of p53 in glioblastoma cells after treatment with X1 embryonic extract (flow cytometry technique).

In fig.2 we compared the activation of p53 in the treated cells with X3 extract to the untreated cells of melanoma.

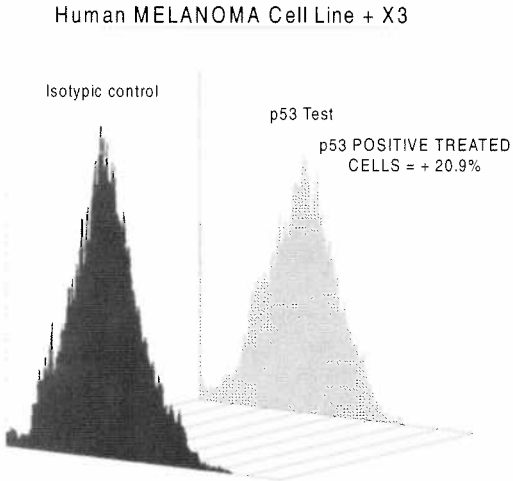

Fig. 2: Activation of p53 in melanoma cells after treatment with X3 embryonic extract (flow cytometry technique).

The treated cells analyzed with flow cytometry technique revealed a 20.9% higher activation. Fig. 3 shows the untreated cells while fig. 4 shows the treated cells with X3 extract of melanoma.

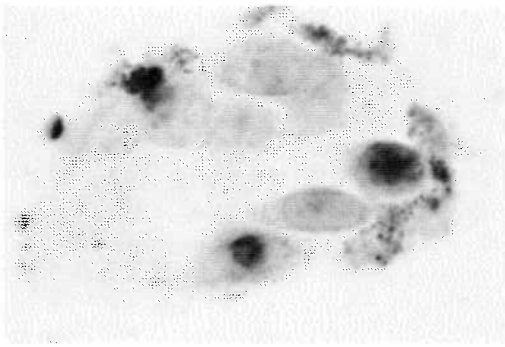

Fig. 3: Melanoma cells prior to the treatment (immunohistochemical method).

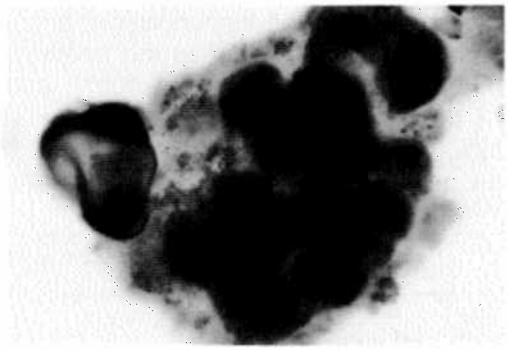

Fig. 4: Melanoma cells after the treatment with X3 embryonic extract (immunohistochemical method).

In both cases the immunohistochemical method was used. Prior to the treatment the high activated cells for p53 were 35%, the medium activated cells were 15%, the low activated cells were 5%, the non-activated cells were 45%. After the treatment the high activated cells were 75%, the medium activated cells were 10%, the low activated cells were 5%, the non-activated cells 10%. Fig. 5 reveals the untreated cells and in fig. 6 the treated cells with X1 extract of hepatocarcinoma.

glioblastoma. The other 4 extracts were ineffective.

Discussion

Our results demonstrate that substances present in the embryo during cell differentiation are able to activate p53 in different in vitro tumor cells. This activation takes place only after the treatment with specific embryonic extracts. Theoretically, we can put forward the hypothesis that each type of tumor is regulated by a specific network of substances present in the embryo during each step of cell differentiation. It is thus possible that the more the tumor is undifferentiated, the sooner the regulators must be taken into the embryo of ovipari (in mammals these regulators could be taken into the uterus wall and into the placenta at the first stages of development). On the other hand, we know that many factors of differentiation are present in the embryo (9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24) and in the decidua of the uterus (25). Some of these factors present in the embryo (26) and in the uterus (27) are able to control the differentiation and the multiplication of specific tumor cell lines. Generally, the factors that cause cell differentiation are represented by a network or by some family of substances that cooperate in several cascades. It is possible that only when the network of factors of differentiation is complete, does the control of multiplication and differentiation of tumor cells take place. On the other hand this point of view is similar to the one concerning the diet/cancer relationship. Today we know that the complex composition of diet with many factors, not just a single factor of diet, play an important role in the prevention of malignancy (28, 29). The microenvironment is very important in controlling the multiplication and the differentiation of normal and tumor cells. The embryo in ovipari and the pregnant uterus in mammals possess the most effective microenvironment in this kind of control. In fact, this microenvironment is quite capable of leading the totipotent stem cells to a complete differentiation. In the course of cell differentiation the administration of known carcinogens cannot induce any tumors in the embryo, probably because the control system of genoma is always active. Recent studies (30, 31, 32, 33, 34) indicate that the function of p53 in the embryo is to prevent malformations.Some authors (35, 36) have therefore defined p53 as the "guardian of the baby", similar to the teratological supressor gene. Nevertheless, when embryonic stress is severe and a large number of mutations are present, p53 cannot repair DNA. In this case, p53 causes the apoptosis of all cells (abortion). These processes also take place in tumor cells when p53 is activated. In this sense tumor cells are similar to embryonic cells affected by many mutations. On the other hand tumor cells and embryonic cells have the same antigens. In fact, besides the known oncofetal antigens, new carbohydrate antigens of Xenopus Laevis embryos shared with human malignant neoplasms have recently been discovered by some authors (37). We think that both embryonic transformed cells and tumor cells can be differentiated by regulators present in the embryo, by passing over the mutations that give rise to malignancy. In the future we will understand more precisely the processes that control the multiplication and the differentiation of embryonic and tumoral cells. It is possible that many other regulatory genes, besides p53, can be activated by embryonic extracts in tumor cells. The present study raises the possibility of further research on embryonic regulators which could be very important in controlling the multiplication and the differentiation of tumor cells. In addition this study may open up a way for the physiological control of malignancy.

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