Mother-Embryo Cross-Talk: The Anti-Cancer Substances Produced by Mother and Embryo During Cell Differentiation. A Review of Experimental Data


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The drawing of a new model based on the rather strict links between embryonic and cancer cells as described in the previous review, is based on a series of experimental evidence gathered during the last 15 years. In this review, the in vitro and in vivo results of all our research work are shown, covering several experimental approaches such as tests on animal models, cytotoxicity assays, and immunohistochemical, flow cytometry and molecular biology methods. The importance of stem cells for our future research is also presented as an intriguing perspective.

Introduction

During pregnancy, the close cross-talk that is formed between the mother and the developing embryo is made of a complex network of molecular factors. This cross-talk is necessary for the prevention of pregnancy-threatening events, including the establishment of abnormally proliferating cell clones, which may damage the integrity of the embryo. This problem had already been encountered by other groups, which referred to activated T-cell clones across the mother-embryo interface (1,2). In the previous review of this, a model that links embryonic development to carcinogenesis, i.e., the onset of an abnormal proliferative program, was described.

According to that model, a cell which survives a series of stochastic mutational events finally evolves into a "stable" gene configuration which leads it to develop a cancerous phenotype. These gene configurations can be quantified in a simple mathematical model, and their number is similar to that of known tumoral types. It is suggested that gene configurations of tumor cells correspond to those of normal embryonic cells, but in tumor cells the multiplication and differentiation programs are uncoupled. A delay of tumor cell proliferation may be achieved by providing tumor cells with the complex network of molecular factors that keep those programs coupled in embryonic development.
The development of a theoretical model of the gene regulation of tumor cells by factors of the embryonic microenvironment comes after several years of work, which involved in vitro and in vivo approaches. In this short review, the results of this work are summarized and shown, giving the reader the experimental bases of the theory presented in the previous paper.

In Vitro Results

In order to assess the effect of the administration of embryonic and decidual extracts on cell proliferation, several tumor cell lines of different origins were used. Zebrafish embryo extracts taken during precise stages of development, i.e., 1000 blastomeres (a full cleavage stage), 50% epiboly (corresponding to the onset of gastrulation), 5 somites and 20 somites were administered to glioblastoma, melanoma, kidney adenocarcinoma, breast carcinoma and lymphoblastic leukemia cells, and proliferation curves were drawn 24 and 48 hours after treatment (3). All cell lines exhibited a slowing down of their proliferation values when treated with extracts from differentiative developmental stages, i.e., from 50% epiboly on. Each cell line had a special slow-down rate and showed a specific response to treatment based on the different developmental stages: for example, the proliferation of glioblastoma was inhibited most by the 50% epiboly stage (named stage I) and least by the 20 somites stage (named stage III), whereas melanoma cells were slowed most by the 5 somites stage (named stage II) and kidney adenocarcinoma cells by stage III. Glioblastoma, melanoma, breast carcinoma and lymphoblastic leukemia cells responded significantly to treatment after 24 hours, whereas kidney adenocarcinoma cells did not exhibit any slowdown at all. After 48 hours however, all cell lines were affected by the treatment, with inhibition percentage values ranging from 73% of the glioblastoma cells and 26% of the melanoma cells treated with stage I extract.

No slowdown response was shown by cells treated with an extract taken from a developmental stage prior to 50% epiboly, namely, the 1000 blastomeres stage (called stage 1k) (3). On the contrary, treated cells exhibited a weak proliferative response. This evidence reinforces our view that differentiative stages of development are characterized by regulatory networks which re-direct tumor cells to a normalized path of differentiation, and that these networks appear from the onset of gastrulation. Before gastrulation, embryos are subjected to merely proliferative stimulating networks which fail to normalize tumor cells, possibly enhancing their abnormal growing potential.

A similar slowing effect on cell growth was observed after the administration of extracts of crude pregnant uterine mucosa to the same tumor cell lines (4). Cells treated with uterine extracts taken from 23 day-pregnant pig exhibited slower proliferation curves, with inhibition percentage values ranging from 80% of the breast carcinoma cells and 67% of the lymphoblastic leukemia cells, to 22% of the glioblastoma cells. As previously observed with zebrafish embryo extracts, each cell line responded to treatment with uterine extracts in a special manner. The day of pregnancy at which the mucosa was collected does not seem to affect the response of tumor cells, since the treatment of glioblastoma cells with uterine extracts taken from pregnant mice on different days led to a diffuse slowing down of the proliferation curve, with inhibition percentage values not significantly different from each other. Finally, the effect of uterine extracts on cell growth appears to be ascribed to tumor cell lines only, since the treatment of a non-tumoral line, murine fibroblast NIH 3T3, did not change the cell proliferation rate.
In order to elucidate which factors are responsible for this effect, we fractionated the whole uterine extract from pregnant pig by low molecular weight cutoffs, and finally we isolated a 5 kDa fraction which retained the slowing efficacy on tumor cell growth. We called this fraction “Life-Protecting Factor” (LPF), because it may contain the molecular factors involved in preserving embryo-mother integrity from pathological cell clones. It is likely that the mechanism of action is apoptosis-mediated, since we observed high levels of a nucleosomal fraction in the medium of tumor cells after 24 hour treatment with uterine extract.

Possible molecular bases of this proliferation-slowing mechanism on cell lines were also investigated by several techniques. Flow cytometry analysis revealed a mean 20% increase of the expression of tumor suppressor p53 in glioblastoma and melanoma cells after treatment with zebrafish embryo extracts (5). Immunohistochemical analysis on treated melanoma and hepatocarcinoma cells showed a dramatic increase of p53 staining compared with untreated cells (5). Not all embryonic extracts were able to induce p53 overexpression, confirming that only precise differentiation stages have the tumor growth-slowing potential. Along with p53, another key-role effector of cell cycle homeostasis, pRb, was shown to be affected by treatment with embryonic factors via the alteration of its phosphorylation state.

**In vivo Results**

The effect of embryonic factors on tumor growth was also observed in vivo by s.c. injection of primary Lewis lung carcinoma cells into C57BL/6J female mice (6). Immediately before injection, tumor cells were challenged with homogenates of 9 day-pregnant mice uteri, non-pregnant uteri, 9 day-old embryos or liver (the latter being a negative control). Only cells that were mixed with the homogenate of pregnant uteri failed to originate primary tumors: the growth rate was nil for the overall experiment time, whereas cells that were mixed with the other homogenates gave rise to primary masses at the same rate as the unmixed cells. Mice injected with pregnant uterus homogenate-treated cells did not develop spontaneous lung metastases at all, whereas all other animals developed metastases, as observed 21 days after the onset of the experiment. Similar results were obtained in another experiment with the administration of homogenates of Drosophila embryos at the blastodermal stage to mice injected with Lewis lung tumoral cells: 15 days after the inoculation of cells, the primary masses were reduced in the treated animals, with a 35% decrease of the tumor weight.

The evidence that the pregnant uterus homogenate abrogated tumor cell growth, whereas the isolated mammal embryo homogenate did not induce any slowing effect, suggested that the factors involved belong to the mother-embryo cross-talk molecular network, and that breaking this interaction abrogates the anti-proliferative effect. Turning to an oviparous model, it was observed that the embryo alone delivers proliferation-slowing factors, although less efficient than those of pregnant uterine mucosa. It may well be that the uterus-embryo system of viviparous animals represents a further evolution of an intrinsic embryonic capability of keeping abnormally proliferating cell clones silent.

**Conclusions**

The *in vitro* and *in vivo* experimental data show that abnormal cell proliferation is somewhat affected by factors that are found in embryos and / or in pregnant uteri. These factors are organized in a network whose complexity should be unscattered to retain its full efficacy. This is particularly true for embryos, whose complex of
molecular factors represents a closed microenvironment that can normalize the behavior of abnormally growing cell populations via a regulatory process involving key-role proteins of cell cycle homeostasis. As for the molecules that characterize mother-embryo cross-talk, they may mediate a more rapid, apoptosis-enhancer process which does not necessarily need the integrity of the network. In fact, LPF represents a low molecular weight fraction of the whole sow pregnant uterine mucosa, and it does slow down the tumor cell proliferation rate alone as well as the whole raw homogenate.

A fundamental aspect of our findings is that only networks present in differentiative stages of embryo development can delay tumor growth, since networks present in multiplicative stages are ineffective or even modulate a slight proliferative effect on cell lines.

Our future research work will be to isolate the single components of these networks. The characterization of each single molecule involved in this kind of regulation will be important to better understand the most subtle mechanisms of action of this networks. An interesting goal will be to focus on the role of stem cells. A recent article shows that the proliferation of neural stem cells is strongly regulated by a homeobox transcription factor, Emx2, the mammalian homologue of *empty spiracles* of *Drosophila*, a fundamental gene for the correct embryonic development (8). This is further demonstration of the strict correlation between embryonic networks of differentiation and stem cells. Stem cells are embryonic cells that can be committed to different cellular types according to the network of factors that constitute the surrounding microenvironment. For example, neural stem cells can be differentiated into cells of the hematopoietic lineage when put into contact with the hematopoietic microenvironment (9), or into skeletal muscle cells when put into contact with factors of skeletal muscle differentiation (10). Similar lines of evidence were observed with stem cells that are other than neural, e.g., liver stem cells (11). Thus, the knowledge of pathways and networks of stem cell differentiation may provide information about the processes of tumor cell differentiation, which is the basis for the normalization of cancer cells to a normal phenotype, by-passing mutations that originate malignancy.

**Bibliography**