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PERSPECTIVES

Perspectives and complexity of an experimental cancer study. The secrets of tumorigenesis

(To the Gupta's, Chaffer's and Weinberg's "perspectives" and to the Nurse's "horizons")

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Abstract: We would like to add to the "mysteriousness", our observations from the application of "identical" BP6 cells either intraperitoneally or subcutaneously. In connection with the concept that tumor development is not only a portrayal of cells proliferation, we could presume that different "environment" will result in structurally different tumors. Morphological differences observed are not significant, but they are present *(Fig. 2, Ref. 12).*
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At present, the description of tumor growth as a mysterious process predominates (1). It is obvious that our knowledge of the principles and process of malignant growth is not and probably will never be complete, as cancer does not represent a single type of disease, but more than 200 distinct diseases. Some recent findings suggest that the principles of tumorigenesis and growth need a "rebuilding". In such a case, it would be very optimistic to assume that we are at the beginning of understanding the cancer process.

The scientific field is in need of an acceptable model to help clarifying what happens during tumorigenesis. The problem is probably the attempt to solve the issue without fully understanding it. We believe that the role of genetic defects in tumorigenesis is overemphasized. If the genetic defects were to influence or determine carcinogenesis and growth, the progress of cancer would be a result of constant proliferation of tumor cells. In this case, the tumor would grow at a constant speed. In experimental tumorigenesis induced by Yoshida tumor cells, it has been demonstrated that the cell growth in the first 6 days is exponential, then a quasi stationary phase follows, and between 14–16 a lethal phase occurs (2). It is known that the tumor growth is a complex process, where the involvement of heterotypic multicellular and multimolecular interactions in the tumor tissue was demonstrated. The process of tumorigenesis in a living organism is composed of several steps, and it could be perceived as some kind of Darwinian evolution on a microscopic level. Though it is not possible to deny the potential role of every single cell or molecule and above all, the importance of the time factor!

Oncological research demonstrates that tumor cells "use" cells of the organism in different modes. Cells in the microenvironment of the tumor are activated or attracted by the tumor cells either directly by released factors or indirectly by the induction of hypoxic environment or necrosis. After the first contact between tumor cells and cells of the host tissue, further progress and growth of cancer will depend on the biological characteristic of tumor cells, as well as the composition of the adherent tissue. Many theories emerged trying to define the tissue reaction in this case. Most probably it is a combination of both factors. The initial reaction of the tissue is mainly immune mechanisms typical for tissue damage. Based on this reaction, tumor cells use mechanisms for their own benefit. The balance between the local production of matrix – metalloproteinases and tissue inhibitors of metalloproteinases determines the intensity of degradation of extracellular matrix. Elevated levels of metalloproteinases expression have been documented in various human malignancies and often closely correlate with high invasion and metastasis of tumor cells (3).

Various cell types (endothelial cells, pericytes, smooth muscle cells, immune cells) participate in angiogenesis, which is crucial for the progression of the tumor. Mast cells support the degradation of tumor extracellular matrix and the invasivity of tumor cells into interstitial stromal tissue, as well as the expansion of the vessel supply and also contribute to immunosuppression. Mice experimental models demonstrated that adenomatous polyps are in-

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filtrated with pro-inflammatory mast cells and that pharmacologic or genetic mast cells depletion is associated with a remission of existing polyps (4). Macrophages are often present in the tumor stroma as so called "tumor associated macrophages" (TAM) with the phenotypic features of M2 macrophages. It was demonstrated that tumor cells migrate towards vessel-associated macrophages and penetrate into the vascular structures only where perivascular macrophages are present (5). Reduction of the amount of TAM was accompanied with an effective suppression of tumor growth and metastasis (6). It appears that other immune cells, particularly neutrophils, increase the metastatic capacity and contribute to tumor cell transendothelial migration (7). Eventually, experimental data indicate that a certain intensity of immune reaction may be necessary for the tumor growth in vivo (8). The presence of neural fibers in tumors represents a structural evidence of the communication between the nervous system and the tumor tissue (9) and indicates the role of higher regulatory systems in addition to surrounding cells. Experimental results demonstrated that nerve endings in tumor tissue release signal molecules that may also support tumor cell proliferation and initiate the formation of metastases. Autocrine and paracrine communication is prominent in the tumor microenvironment between cells through variety of growth factors, cytokines, interleukins and other mediators, resulting in regulating the activity of intracellular signaling pathways.

We believe that the term "the mysterious steps" in carcinogenesis (1) as well as in tumor biology fitted very well. "Mysterious" can be also used to describe the existence of "special cells", which were observed in the population of tumor cells, and act like stem cells and therefore are known as "cancer stem cells" (CSC). They have the ability to replicate. After transplantation to an appropriate recipient they can reconstitute a tumor. It is suggested, that these very cells are responsible for tumor growth and metastasis (10). It seems that their presence is an indicator of the degree of progression and genetic instability of the tumor. Bearing in mind the experimentally observed differences between host organisms and types of cancer, a question arises concerning the plasticity of CSC and their mysterious significance in the overall tumor growth (11). The study of CSC biology is based on the determination of CSC in tumors. And of course, an ideal model that would simulate the course of tumor growth is needed. We fully agree with the opinion of the authors Gupta et al (2009) that "CSC numbers cannot presently be stated in absolute terms, but only relative to the animal model used to measure CSC representation" and "this limitation will probably need the adoption of several animal host models, each of which will recapitulate a distinct tissue microenvironment present at a specific stage of human tumor progression".

Cancer ethiopathogenesis research uses large amounts of human (in vitro) and animal (in vitro, in vivo) models. In animal experiments of cancer biology, it is necessary to take into account the constraints associated with the application of their findings to human medicine. There are significant differences (genetic, metabolic, ontogenetic and other) that affect the cancer pro-

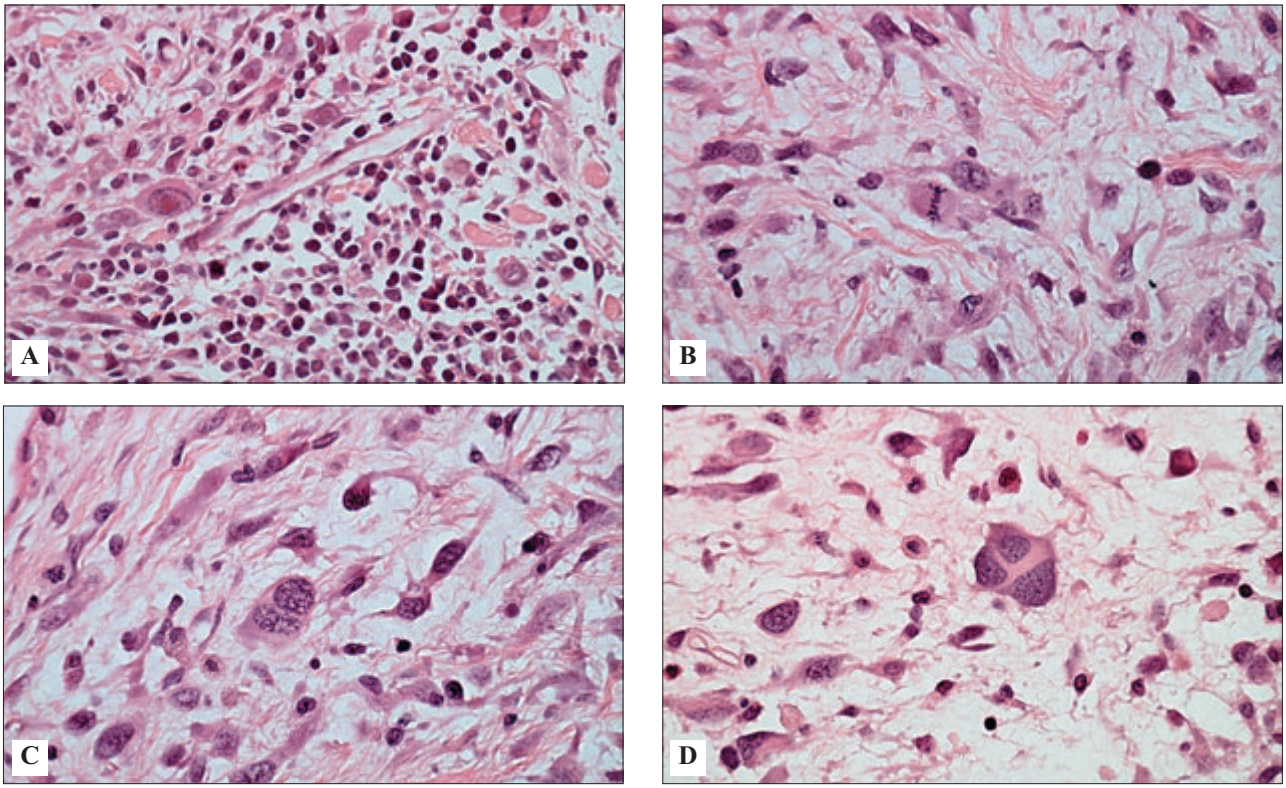

Fig. 1A (SC): pleomorphism of tumor cells and their nuclei, abosphilia and eosinophilia of cell cytoplasm, blood vessels sporadically present surrounded by lymphocytes, 1B (SC): frequent typical and atypical mitosis in tumor tissue, nuclei pleomorphism, 1C (SC), D (IP): nuclei pleomorphism, huge tumor cells with large nuclei, numerous nucleoli and minimum cytoplasm.

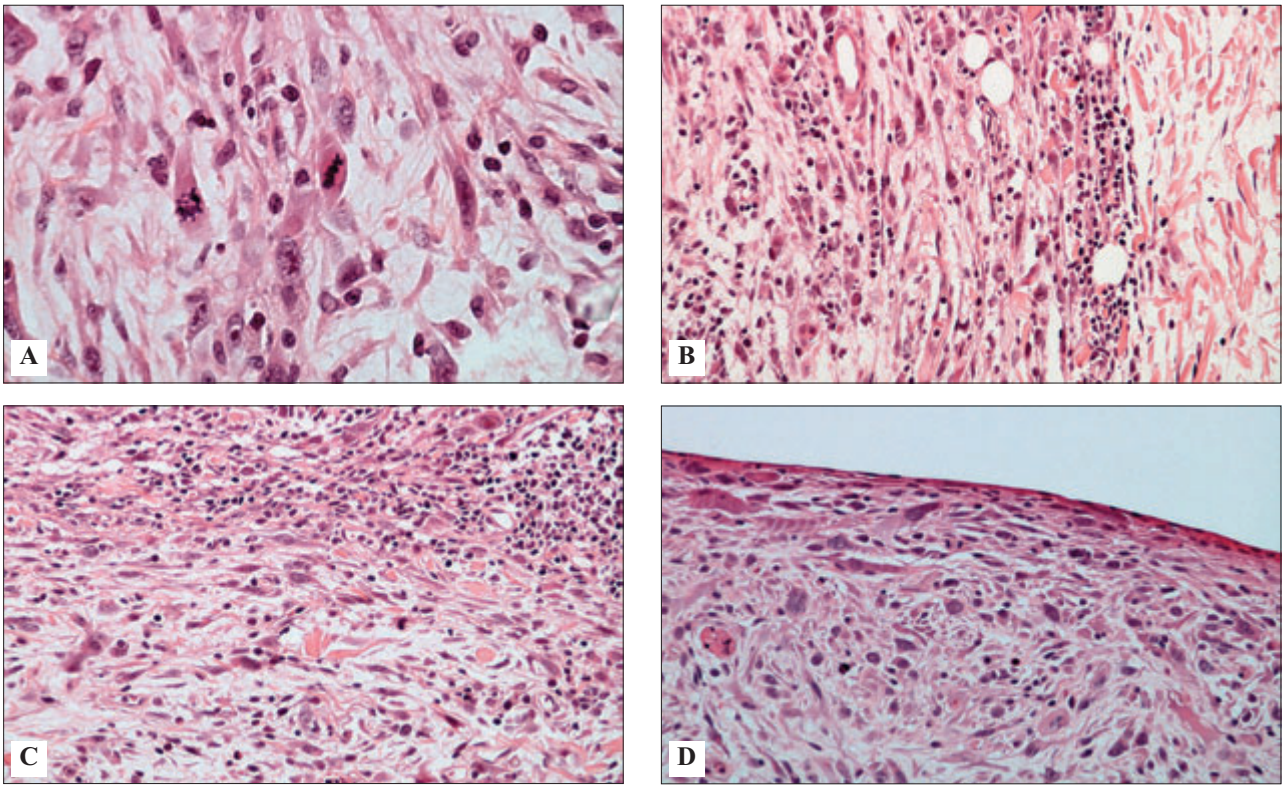

Fig. 2A (SC): frequent mitosis, small optically empty vacuoles in the cytoplasm, B (SC): sporadic blood vessels in the tumor tissues, lymphocyte infiltration of tumor tissue, mainly the borderline zone, C (SC): dense lymphocyte infiltration of the tumor periphery, D (IP): flat mesothelial cells surronding intraperitoneal BP6 tumor.

cess in laboratory animals compared to humans. Despite the distinct differences, there are other mechanisms that have shown to be almost completely identical. Animal studies provide valuable information in terms of complex interactions between organism and tumor tissue. Tumor cell application into the recipient animal's body has a few unresolved limitations in terms of "successful development of cancer", therefore we suppose that this technique is suitable for the detection and revealing of mysterious events associated with tumor growth.

Mysterious events in our model of experimental tumorigenesis

During the monitoring of tumorigenesis using a rat model and BP6 tumor cell line, we documented several observations that could be considered as mysterious. The question: what happens during the time between application tumor cells and the appearance of the tumor comes into consideration. We dedicated a great deal of interest in studying the dynamics of tumor growth.

In experimental tumorigenesis we need to successfully induce tumors in the majority of experimental animals. In order to obtain the precisely defined model we found that the application of a smaller amount of tumor cells does not provide "adequate" results in terms of the incidence of tumors in comparison to animals with larger amounts of injected tumor cells. It is assumed that lymphocytes are responsible for the elimination of tumor cells after their application to animal models. In the early stages of tumorigenesis, "dangerous" signals are absent, because only few cells die and therefore is processed by antigen-presenting cells. The entry of inactive lymphocytes into tumor tissue is not effective. Only when the tumor reaches a certain size and apoptosis occurs, "dangerous" signals appear. This can explain why the immune response occurs late. On the other hand, BP6 cells are foreign cells for the animals. Nevertheless, they "overcome" all barriers of the host organism. We believe that there are certain "critical borderline points" for a certain amount of cells that determines the successfulness of tumor induction by the application of tumor cells to experimental animals.

These observations suggest a hardly comprehensible complexity of tumor growth. We assume that during the proliferation of tumor cells, there are certain aspects are not solely determined by duration and sequence. Complexity as a characteristic is adds up in time, but it is always a non-linear process. That is why the process can be considered as the one with signs of complexity.

We would like to add to "mysteriousness" our observations from the application of "identical" BP6 cells either intraperitonealy (IP) or subcutaneously (SC). In accordance with the concept that tumor development is not only a portrayal of cells proliferation, we could presume that different "environment" will result in structurally different tumors. Morphological differences observed are not significant, but they are present.

Our observation did not show a distinct variation in the structure of the tumor tissue itself. Subcutaneous and intraperitoneal

tumors were formed as solid structures by cells of different sizes and shapes (radial, fusiform, spherical, and sometimes bizarre shapes) (Fig. 1A). Cell nuclei were present in varying numbers, with different sizes, varying from small with conspicuous density of nuclear chromatin, through larger with medium to high chromatin content. A significant diversity of the nuclei shapes was also documented – from small round to spindle, lobed, and sometimes totally bizarre (Figs 1A–1D). In histological sections, typical and atypical mitosis were frequently observed (Figs 1B, 2A). Huge tumor cells contained giant nuclei with numerous nucleoli and a minimum amount of cytoplasm (Figs 1C, 1D). Cytoplasm of tumor cells was sometimes basophilic and sometimes eosinophilic (without nucleus) (Fig. 1A). Numerous, optically empty, and very small vaccuoles were observed in the cytoplasm, often near the nuclei (Fig. 2A). Some cells contained homogeneous cytoplasm. In both tumor types, small blood vessels (mainly capillaries and venulles) were sporadically present (Figs 1A, 2B).

Surprising differences were observed where tumor tissue contacted the surrounding area in organism. In the case of subcutaneous tumor, the margin between the tumor mass and the dermis was pronounced, the tumor was not capsulated. In the surrounding dermis, several enlarged arteries and veins could be seen. Fat cells and lymphocytes markedly infiltrated the surrounding dermis (Fig. 2C). Dense lymphocyte infiltration was present on the periphery of the tumor (Figs 2B, 2C) (small lymphocytes with dark nuclei, without cytoplasm with cytotoxic T lymphocytes appearance). This picture indicates an ongoing anti-tumor activity of immune cells. In the tumor itself some of the lymphocytes were in contact with the surface of tumor cells, some lymphocytes were in contact with tumor cell cytoplasm. And, numerous lymphocytes also occurred around blood vessels (Fig. 1A). Intraperitoneal tumor was histologically similar to the subcutaneous but its surface was “isolated”, covered with flat mesothelial cells (Fig. 2D).

The observed histological pictures indicate different reactions of the environment to tumor cells. The environment, in which BP6 cells proliferate after subcutaneous and intraperitoneal application, is different. It is therefore natural that the composition of tumors will differ, although, not fundamentally. Therefore tumorigenesis as process depends on the “aggressivity of cancer cell proliferation” and the local conditions “the environment”, as well as the entire host organism. Our assumptions are distorted with the observation that in some subcutaneous tumors, there was a rapid growth, then involution and a reduction in size. Cancer research aims to “fight” against cancer process in the organism. Perhaps the understanding of the biological significance of tumor could help in the sense that in future we would try to create conditions in the organism that favor tumor formation resulting in the formation of a “tumor organ” with differentiated and less aggressive cells.

Conclusions

In one of his rare books, Arnold Katz wrote a funny note: “dear all, the inside of the cell is not a vegetable soup”. By that he certainly wanted to express that inside of cells is not the reservoir, nor the storehouse of all kinds of molecules. Taking into account that many molecular processes oscillate with various frequencies and intensities, the relations between oscillating processes become difficult to comprehend. Molecular bonds most likely work as logic modules. They do not operate isolated, rather in various combinations. It is likely they can operate as the switches of the oscillators with an own memory. However switching on and off are not “all” options available. These two “extreme conditions” would not be able to provide complex functions. Slowing of the processes can create another situation in a complex oscillation phenomena.

Perhaps we could assume that the coupling of protein molecules, which operate as automatism is evolutionarily the oldest. Some can act as “hubs” in a computer network that has memory (12). To understand the complex, mysterious process, the use of fluorescent proteins will be needed to observe not only the movement but also the involvement of protein molecules in complex processes. Physical manipulation (e.g. nanoparticles) aiming to cause changes in cell behaviour appears to be very promising in cancer research, where targets of manipulation are proteins involved in paracrine communicating signals.

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