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Systems Biology of Tumor Dormancy: linking biology and mathematics on multiple scales to improve cancer therapy

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Abstract

For many decades it has been appreciated that tumor progression is not monotonic, and development of a cancer cell does not equate to inevitable cancer presentation in the clinic (1). Tumor progression is challenged by numerous intrinsic and extrinsic bottlenecks that can hold the tumor in dormant stages for prolonged periods. Given the complex, multi-scale nature of these bottlenecks, the Center of Cancer Systems Biology organized a workshop on critical issues of systems biology of tumor dormancy. The program for the meeting this past July, chaired by Nava Almog and Heiko Enderling, included discussions and interactive breakout sessions on regulation of tumor dormancy by angiogenesis, tumor-immune system interactions, cancer stem cell kinetics, and cell signaling pathways. Three important conclusions emerged from the meeting. The first was the urgent need to differentiate between tumor cell and tumor population dormancy of the primary tumor and metastatic deposits, the second was the continued need for interdisciplinary dialogs, and the third was the need to bring cross-scale mechanistic thinking to the field to achieve a more robust understanding of tumor dormancy and its clinical implications.

Introduction

Given the extent to which solid tumors remain asymptomatic at small sizes, marked by balanced cell proliferation and cell death, we know that cancers can exhibit dormancy under conditions that transcend many tumor-specific molecular and physiological details (2–4). Understanding how dormancy is controlled would therefore be an invaluable augment to current treatment modalities. The question about underlying kinetics and systems-level regulation of tumor dormancy was the title and aim of the inaugural workshop, sponsored as part of the Educational and Outreach effort of the Integrative Cancer Biology Program of the National Cancer Institute. Nine invited lecturers from the fields of medicine, biology, and mathematics were selected to represent different scientific backgrounds. These lectures, summarized below, included paradigm descriptions of major known regulation pathways of tumor dormancy: tumor angiogenesis, systemic immune response, molecular and cellular signaling pathways, and cancer stem cells.

Angiogenesis and tumor dormancy

A search for the molecular regulators and underlying mechanisms of primary tumor dormancy using unique experimental models of human tumor dormancy was described by **Nava Almog** (Tufts University, Boston, MA, USA). These models include pairs of cell lines, derived from human breast carcinoma, glioblastoma, osteosarcoma and liposarcoma cell lines. In each pair, one cell line forms dormant micro-tumors while the other forms fast growing ones when injected into immune-deficient mice. The micro-tumors remain occult and microscopic (diameter of only 1–2 mm) at the site of injection for a prolonged period of time, until they eventually spontaneously switch and initiate a rapid mass growth phase. The dormancy phase is associated with constant proliferation of tumor cells, balanced by

apoptosis. In these models, impaired angiogenesis is one of the hallmarks of dormant micro-tumors (5, 6). These models have been used for comparison of gene expression profiles between dormant and fast growing tumors, which resulted in the identification of genes and microRNAs that are uniquely expressed only in dormant tumors or only after the switch to rapid growth (3, 7). The consensus gene signature of human tumor dormancy was generated by identifying all the differentially regulated genes that had the same expression patterns in all the tumor types analyzed.

Additional insights into the regulation of tumor dormancy by angiogenesis in experimental tumors were presented by **Stefano Indraccolo** (Istituto Oncologico Veneto, Padova, Italy). He discussed the hypothesis that signals stemming from angiogenic endothelial cells regulate the behavior of dormant cancer cells. His group previously demonstrated that the Notch ligand Dll4, induced by angiogenic factors in endothelial cells, triggers Notch3 activation in neighboring tumor cells and promotes a tumorigenic phenotype (8–11). Recently, they strengthened evidence that Notch3 signaling is involved in tumor dormancy and observed that MKP-1 levels – a broadly expressed phosphatase – are controlled by Notch3 by regulation of protein ubiquitination and stability. Notch3 and MKP-1 levels are consistently low in dormant tumors, and this is accompanied by relatively high levels of phosphorylated p38, a canonical MKP-1 target. These results elucidate a novel angiogenesis-driven mechanism involving the Notch and MAPK pathways that controls survival of T-ALL cells and tumor dormancy (12).

Using data from 420 untreated clinically apparent human breast cancers, **Philip Hahnfeldt** (Tufts University, Boston, MA, USA) was able to reconstruct a consensus tumor-vascular dynamic underlying tumor growth. He found that tumors first expand their host support, i.e., their ‘carrying capacity’, well in advance of their growth (13). Then, as tumors continue to grow, they actively curtail host support, defining a theoretical point of post-vascular dormancy that may or may not be reached over the course of disease. These dynamical findings counter past thinking that the carrying capacity comprises a fixed support level to which tumor growth will theoretically asymptote (e.g., by following Gompertz growth). Instead, an active control of host support by the tumor is demonstrated; one that shows striking similarity to that seen in avian and mammalian embryogenesis. The idea that tumors, despite their variability, may rely on co-opted host programs offers new insights into the carcinogenesis process and a potential avenue for therapeutic exploitation.

Immune response and tumor dormancy

The role of the immune response in maintaining tumors in a microscopic, asymptomatic state was discussed in a series of presentations. **Bruno Quesnel** (INSERM, Lille, France) described the long-term survival of dormant tumors within a hostile environment. Dormant tumor cells may persist as quiescent cells, or they may develop active survival mechanisms by which a substantial proportion of cellular death among residual cancer cells is compensated for by active replication (an equilibrium). The equilibrium between immune response and tumor cells can lead to long-term tumor dormancy. For example, in the DA1-3b model, tumor cells escape dormancy by becoming resistant to cytotoxic lymphocytes (CTLs) (14). Dormant tumor cells may over-express B7-H1 and inhibit CTL-mediated lysis. Toll-like receptor (TLR) ligands induce B7-H1 expression and may help dormant tumor cells to escape the T-cell response (15). Dormant tumor cells may also resist apoptosis by deregulation of survival pathways like JAK/STAT and AKT/mTOR, and by paracrine production of cytokines. These observations support an active resistance of tumor cells to the killing effects of the immune system. Such mechanisms of immunoevasion may also lead to cross-resistance to various anti-cancer agents, suggesting that tumor dormancy by itself could limit treatment efficacy.

Kathleen Wilkie (Tufts University, Boston, MA, USA) provided an overview of mathematical models of tumor-immune interactions using ordinary differential equations with emphasis on immune-induced primary tumor population dormancy. Discussion started from basic Lotka-Volterra or 'predator-prey' type models, in which tumor growth stimulates an immune response that in turn reduces the tumor population. This simple model can easily be extended to incorporate a more detailed description of the immune system. The presented model featured common mathematical expressions representing biological behaviors such as tumor growth rate, immune predation rate, and immune recruitment rate. The talk concluded with a summary of her new ideas to incorporate both tumor-promoting and tumor-inhibiting immune actions into a generalized mathematical modeling framework analogously to Hahnfeldt et al., 1999. Tumor dormancy, or mathematically the attainment of a non-zero steady state or plateau, can be observed after damping oscillations in tumor size.

Signaling pathways, cancer stem cells, and tumor dormancy

Accumulating studies show that tumors are heterogeneous populations of cancer stem cells and non-stem cancer cells. Cancer stem cells can initiate, maintain, and reinitiate new tumors whereas large numbers of non-stem tumor cells cannot.

Heiko Enderling (Tufts University, Boston, MA, USA) presented a theoretical model framework in which individual cells, cancer stem cells, and non-stem cancer cells interact with each other and their immediate environment and over time form solid primary tumors (16). For large areas of the underlying parameter space, tumor dormancy was shown to be the predominant phenomenon. Non-stem cancer cells rapidly become the dominant population in a tumor and force the small number of cancer stem cells into quiescence. Because of limited proliferation capacity of non-stem cancer cells, the tumor population eventually ceases to expand and is held to a macroscopically non-advancing size, subject to oscillations in cell number due to cells dying at the outer rim and previously quiescent cells becoming proliferative again. These primary tumor populations can remain non-advancing for many years or decades. Only when cell kinetics combine in an unexpected manner – i.e., a large migration capacity of cancer stem cells, low non-stem cancer cell proliferation capacity, and frequent non-stem cancer cell death – can the cancer stem cell pool sufficiently expand to drive tumor progression beyond dormancy (17).

The molecular mechanisms underlying cellular dormancy of disseminated tumor cells (DTCs) and the microenvironment control of dormancy timing was described by **Julio Aguirre-Ghiso** (Mount Sinai School of Medicine, NY, NY, USA). In a model of spontaneous dissemination of head and neck squamous carcinoma (HNSCC), cells disseminating to different organs have striking differences in dormancy periods and molecular characteristics. While metastases in the lungs grow after a dormancy period of 2–3 weeks, cells that metastasize to bone marrow (BM) remain occult and rarely grow. The dormant phenotype of the BM DTCs is associated with a strong activation of the stress activated kinase p38α/β, low activation of extracellular signal regulated kinase (ERK1/2). This results in induction of the transcription factors p53, NR2F1 and BHLHB3 and cell cycle inhibitors such as p21, p27, and p15. The key regulator of dormancy, which mediates signals from the microenvironment, is p38α/β and evidence was presented that TGFβ2 might be a potential inducer of this phenotype in sites like the BM. While p38α/β activation initiates a quiescence program to induce and maintain dormancy, its inhibition results in accelerated metastases and an increased repertoire of organs that support DTC expansion (18).

Furthermore, a computational approach identified a p38α/β -dependent transcription factor network (19) that regulates tumor cell quiescence. The genes in this network, which

included those mentioned above, were used to generate a dormancy scoring system that when applied to gene expression profiles from clinical samples of breast cancer stratified ER + patients into those prone to early or late development of metastasis. This work could be useful for predicting whether a patient might carry occult residual breast cancer in dormant or progressing states.

Dean Felsher (Stanford University, Palo Alto, CA, USA) discussed the crucial role of oncogene activation in tumor progression and the implications on tumor dormancy. An inducible transgenic model in which myc activity is regulated by tetracycline was applied in several cancer experimental models (20). Turning myc off during lymphomagenesis resulted in induction of cell cycle arrest, differentiation, and apoptosis. In highly metastatic osteosarcoma, inactivation of myc also resulted in induction of differentiation and senescence. In invasive hepatocarcinoma, tumor cells stayed dormant as long as myc remained inactivated, while its reactivation restored a neoplastic phenotype. In general, although the consequences of myc inactivation are different for each tumor type, cell cycle arrest, apoptosis, and differentiation/senescence appear to be common mechanisms (21). Interestingly, myc inactivation revealed stem cell-like properties, as it blocked self-renewal of tumor cells. Tumor response to oncogene inactivation also depended on the interaction of tumor cells with their microenvironment and the host, and in particular the immune system. In double conditional transgenic models, tumor regression depended upon the specific tissue context. In a T-cell lymphoma model, tumor regression following myc inactivation was associated with cellular senescence, angiogenesis inhibition, and chemokine expression mediated by CD4 T cells.

A connection between the immune system, stem cell signaling pathways, and tumor dormancy was proposed by **Tobias Schatton** (Harvard Medical School, Boston, MA, USA). A subpopulation of malignant melanoma was identified as tumor-initiating cells based on expression of the chemoresistance mediator ABCB5 (22). In vivo genetic linage tracking was used to prove self-renewal and differentiation capacity of ABCB5+ cells. Many of the biological mechanisms involved in controlling the tumor dormant state affect this cellular population behavior, including cell cycle modifications, alteration of angiogenic processes, and modulation of antitumor immune responses. While frequency of ABCB5+ cells correlated with disease progression, selective targeting of this cellular population using systemic administration of a monoclonal antibody resulted in inhibition of tumor formation and growth. Promotion of tumor growth by these cells is also associated with VEGF-R1 expression and vasculogenic mimicry. This might explain the survival of this tumor fraction despite poor vascularization. Interestingly, the ABCB5+ melanoma initiating cells can also modulate the anti-tumor immune response, establishing immunomodulatory functions to cancer stem cells (23). Clear similarities between properties of dormant tumor cells and this population of melanoma initiating cells exist, such as the ability to initiate tumor growth, the preferential ability to survive tumor therapy, and the long-term persistence which ultimately causes delayed cancer recurrence and metastatic progression.

Summary and conclusions

In the meeting, tumor dormancy was discussed according to the operational definition of a period during which growth of a tumor population is halted but cells within the population remain viable (1, 4, 24, 25). In the case of a single cell, either circulating in the blood system or solitary at a secondary site, this can also be described as quiescence. On the population level, whilst all cells in a tumor may be quiescent, dormancy phases can be observed when cell proliferation and cell death are balanced – either by intrinsic decision making or environmentally enforced conditions. The discussions during this meeting highlighted the variety of interpretations of dormancy, and future discussions will be necessary to

understand the different mechanistic regulators of tumor dormancy on the cell or population levels of the primary tumor, as well as at the metastatic site.

The fruitful scientific exchange at this workshop emphasized the value of and the continued need for interdisciplinary collaboration to better understand tumor dormancy. Biologists, clinicians, and mathematicians should combine their expertise to identify the underlying kinetics of and environmental contributors to the dormancy phenomenon.

It became clear that angiogenesis-related dormancy, the immune system, stem cells, and perhaps other mechanisms, must interact to maintain tumors in a non-advancing, pre-clinical state. Although this meeting focused in depth on dormancy of the primary tumor or disseminated cancer cells, it was appreciated that a tumor can also alter the growth rate and progression of other tumor populations elsewhere in the body. Extending on this observation, the interdisciplinary research conducted at this meeting pointed to the likely existence of other overarching principles of tumor dormancy that stand to redefine our thinking about this fundamental process.

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