Meeting Report

Systems Biology of Tumor Dormancy: Linking Biology and Mathematics on Multiple Scales to Improve Cancer Therapy

Heiko Enderling, Philip Hahnfeldt, Lynn Hlatky, and Nava Almog

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. Tumor progression is challenged by numerous intrinsic and extrinsic bottlenecks that can hold the tumor in dormant stages for prolonged periods. Given the complex, multiscale 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 N. Almog and H. 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. Cancer Res; 72(9): 2172-5. ©2012 AACR.

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 physiologic details (1–4). 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 education and outreach effort of the Integrative Cancer Biology Program of the National Cancer Institute (Bethesda, MD). Nine invited lecturers from the fields of medicine, biology, and mathematics were selected to represent different scientific backgrounds. These lectures, summarized later, 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 School of Medicine, Boston, MA). 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 microtumors whereas the other forms fast growing ones when injected into immune-deficient mice. The microtumors 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 microtumors (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 miRNAs 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 showed 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 mitogen-activated protein kinase

Authors’ Affiliation: Center of Cancer Systems Biology, St. Elizabeth’s Medical Center, Tufts University School of Medicine, Boston, Massachusetts

Corresponding Author: Nava Almog, St. Elizabeth’s Medical Center, Tufts University School of Medicine, 736 Cambridge Street, CBR-112, Boston, MA 02135. Phone: 617-779-6532; Fax: 617-562-7142; E-mail: nava.almog@tufts.edu

doi: 10.1158/0008-5472.CAN-11-3269

©2012 American Association for Cancer Research.
phosphatase-1 (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 School of Medicine, Boston, MA) was able to reconstruct a consensus tumor vascular dynamic underlying tumor growth. He found that tumors first expand their host support, that is, 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 postvascular dormancy that may or may not be reached over the course of disease. These dynamic 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 shown; 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 in equilibrium with active replication. 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 CTLs (14). Dormant tumor cells may overexpress 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 Janus-activated kinase (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 immune evasion may also lead to cross-resistance to various anticancer agents, suggesting that tumor dormancy by itself could limit treatment efficacy.

Kathleen Wilkie (Tufts University School of Medicine, Boston, MA) 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 biologic 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 analogous to that used by Hahnfeldt and colleagues (13). Tumor dormancy, or mathematically the attainment of a nonzero steady state or plateau, can be observed after damped 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 nonstem cancer cells. Cancer stem cells can initiate, maintain, and reinitiate new tumors whereas large numbers of nonstem tumor cells cannot.

Heiko Enderling (Tufts University School of Medicine, Boston, MA) presented a theoretical model framework in which individual cells, cancer stem cells and nonstem 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. Nonstem 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 nonstem cancer cells, the tumor population eventually ceases to expand and is held to a macroscopically nonadvancing 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 nonadvancing for many years or decades. Only when cell kinetics combine in an unexpected manner—that is, a large migration capacity of cancer stem cells, low nonstem cancer cell proliferation capacity, and frequent nonstem 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 (DTC) and the microenvironment control of dormancy timing was described by Julio Aguirre-Ghiso (Mount Sinai School of Medicine, New York, NY). 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. Whereas metastases in the lungs grow after a dormancy period of 2 to 3 weeks, cells that metastasize to bone marrow remain occult and rarely grow. The dormant phenotype of the bone marrow DTCs is associated with a strong activation of the stress-activated kinase p38α/β and a low activation of extracellular signal–regulated kinase (ERK1/2). This results in induction of the transcription factors p53, NR2F1, and BHLH3 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 bone marrow. Whereas p38c/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 p38c/β-dependent transcription factor network (19) that regulates tumor cell quiescence. The genes in this network, which included those mentioned earlier, were used to generate a dormancy scoring system that when applied to gene expression profiles from clinical samples of breast cancer stratified estrogen receptor (ER)þ 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 Felscher (Stanford University, Palo Alto, CA) discussed the crucial role of oncopgene 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 experimental cancer 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, whereas 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 oncopgene 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). A subpopulation of malignant melanoma was identified as tumor-initiating cells based on expression of the chemoresistance mediator ABCB5 (22). In vivo genetic lineage tracking was used to prove self-renewal and differentiation capacity of ABCB5þ cells. Many of the biologic mechanisms involved in controlling the tumor dormant state affect this cell population behavior, including cell-cycle modifications, alteration of angiogenic processes, and modulation of antitumor immune responses. Increased frequency of ABCB5þ cells correlated with disease progression, and selective targeting of this subpopulation 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 vasulogenic mimicry. This might explain the survival of this tumor fraction despite poor vascularization. Interestingly, the ABCB5þ melanoma-initiating cells can also modulate the antitumor immune response, establishing immunomodulatory functions of 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 might ultimately cause 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, while 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 nonadvancing, preclinical 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.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors’ Contributions

Conception and design: H. Enderling, N. Almog
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): N. Almog
Writing, review, and/or revision of the manuscript: H. Enderling, P. Hahnfeldt, L. Hlatky, N. Almog

Grant Support

The annual Center of Cancer Systems Biology workshop series (http://cancer-systems-biology.org/workshop.html) is funded by the NCI ICBP program under Award Number U54CA149233. We also acknowledge support from the National Brain Tumor Society, the Society for Mathematical Biology, and Springer US for this inaugural meeting.

Received September 29, 2011; revised February 23, 2012; accepted March 8, 2012. Published OnlineFirst March 13, 2012.
References

14. Saudemont A, Quesnel B. In a model of tumor dormancy, long-term persistent leukemic cells have increased B7-H1 and B7.1 expression and resist CTL-mediated lysis. Blood 2004;104:2124–33.

Published OnlineFirst March 13, 2012; DOI: 10.1158/0008-5472.CAN-11-3269
Systems Biology of Tumor Dormancy: Linking Biology and Mathematics on Multiple Scales to Improve Cancer Therapy

Heiko Enderling, Philip Hahnfeldt, Lynn Hlatky, et al.


Updated version  Access the most recent version of this article at:
doi:10.1158/0008-5472.CAN-11-3269

Cited articles  This article cites 25 articles, 12 of which you can access for free at:
http://cancerres.aacrjournals.org/content/72/9/2172.full#ref-list-1

Citing articles  This article has been cited by 1 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/72/9/2172.full#related-urls

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.