Beyond the Cancer Cell: Progression-Level Determinants Highlight the Multi-Scale Nature of Carcinogenesis Risk

Lynn Hlatky* and Philip Hahnfeldt

Center of Cancer Systems Biology, GRI, Tufts University School of Medicine, Boston, MA 02135

*To whom correspondence should be sent:

Lynn Hlatky, PhD
Director, Center of Cancer Systems Biology
Genesys Research Inst.
Tufts University School of Medicine
736 Cambridge St., CBR-112
Boston, MA 02135
Tel: 617-789-2997
Fax: 617-562-7142
Abstract:
Over the last several decades, improved awareness of the prevalence of carcinogens in the environment, along with a growing appreciation of the complexity of the carcinogenesis process, has shifted policy on cancer risk from one of strict avoidance of carcinogens to one of adherence to exposure limits deemed ‘safe’ based on quantitative risk estimation. Meanwhile, given the mutagenic nature of most carcinogens, attention has gravitated to developing a genetic rationale for measuring and comparing risks. This focus has culminated in the now well-established multistage mutational paradigm, which holds that a stepwise sequence of mutations drives cell “initiation” and the subsequent “transformation” of an initiated cell into a cancer cell, and that, once created, a cancer cell will inevitably undergo “progression” to become overt disease. Relatively unappreciated is the effect progression-phase population- and tissue-level bottleneck events may have on this process. Attesting to this is the prevalence of tumor dormancy, a state of arrested growth of an otherwise fully malignant, often microscopic cancer mass, maintained by interactions among cancer cells and between cancer and host cells. The proper inclusion of such progression-modifying influences would clearly behoove risk estimation, and improve our understanding of the natural history of cancer by accounting for the less-than-certain risk of eventual cancer disease even when cancer cells are present. Such an improved understanding, in turn, stands to better inform policy-making and influence such clinical practice decisions as whether to treat the increasingly smaller tumors detectable with advancing technologies.
The issue of cancer risk from exposure to environmental carcinogens impacts millions of individuals worldwide. Policy considerations include the setting of appropriate standards for exposure in the workplace, home and clinic, along with assessing tradeoffs among alternative energy sources and classifying carcinogens to which the public may be exposed. Of special interest to the U.S. Dept. of Energy (DOE) is the cancer risk associated with the post-Cold-War cleanup of nuclear waste; more generally, the longstanding question of whether there exist radiation dose or dose-rate thresholds below which there is no increase, or perhaps even a decrease, in cancer risk (1, 2). The National Aeronautics and Space Administration (NASA) seeks to estimate the excess radiogenic cancer risk from long-duration space missions (3, 4). In response to workplace concerns, the National Institute for Occupational Safety and Health (NIOSH) upgraded an earlier policy that called for “no detectable exposure levels for proven carcinogenic substances” (5). It now recommends chemical carcinogen exposure limits go beyond ‘no effects’ to include exposure levels at which there may be residual risks. RISC-RAD, an 11-country project funded by the European Commission, is dedicated to understanding carcinogenesis risk “at low doses and dose rates and the effects of radiation quality at exposures below those at which direct information is available”. With the now widespread implementation of airport x-ray backscatter screening procedures and the high-profile Fukushima nuclear power plant disaster, considerable public attention has been drawn to these issues.

In parallel with these developments, efforts have been underway to fully understand the events leading to cancer cell generation. Carcinogenesis, the sequence of events that convert a normal cell into an expanding, invasive neoplastic mass, is commonly modeled as initially involving a series of cell mutations followed by clonal expansions (6, 7). These so-called “natural history” models of carcinogenesis take on various forms, but are inspired by the basic two-stage mutational paradigm. By this paradigm, a chance DNA-altering event (e.g., a point mutation, deletion, translocation, copy number change, or heritable epigenetic alteration) “initiates” a cell, i.e., alters its growth characteristics and predisposes it for later carcinogenic conversion. In a phase called “promotion”, the progeny of this initiated cell go on to display modified growth characteristics and altered propensities to undergo further spontaneous or induced genetic change. This cycle of DNA alteration and clonal expansion is revisited a number of times. Ultimately, a cell in the clone undergoes a “transformation” event, becoming a frank cancer cell, which expands within the host tissues to become overt disease (Figure 1).
As carcinogenesis proceeds, what begins as a cell-level series of events graduates to include population-level effects shortly after initiation, then comes to include tissue-level influences as the nascent tumor grows and acquires larger-scale properties e.g. invasiveness and metastatic potential, upon interaction with the host.

Work in colon carcinogenesis has refined our understanding of the malignant transformation event. It is known, for instance, that sequential mutations of key genes, adenomatous polyposis coli (APC), Ras/Raf, and p53, can transform a colonic crypt cell (8). After a cancer cell is created, the progeny population evolves over a phase termed progression, during which a number of the characteristic features of cancer, e.g., tumor angiogenesis, resistance to cell death and insensitivity to growth-inhibitory signaling, are acquired or enhanced (9). But the rapid accumulation of these and other molecular insights into carcinogenesis gained over the last 50 years would only gradually win favor for mechanistic risk modeling to become the guide for public policy on carcinogen exposure. With the renaissance in attitude that took place in the late 1970’s, a ‘precautionary principle’ that arbitrarily sought exposure limits well below the No Observed Adverse Effect Level (NOAEL), finally gave way to a ‘quantitative risk assessment’ objective that made experimentation and elucidation of molecular mechanism the gold standard (10).

But while the embrace of molecular mechanism for risk-based policymaking is now the mainstay, unexpected challenges to the carcinogenesis paradigm itself have begun to surface. As a case in point, research into cancer risk from radiation exposures in the low-dose realm is tackling the perplexing question of whether the risk at very low doses remains dose-proportional (11), or instead deviates higher (12) or lower (13), perhaps to the point where the risk is actually reduced (14). However this plays out, these investigations have served to bring to the risk modeling forefront a growing sentiment that the multistage mutational paradigm, with the rather simplistic monotonic association it makes between cellular DNA damage and risk, neglects contributions from other biological scales of influence.

Complicating matters further, mechanistic insights into carcinogenesis are frequently derived indirectly from the study of cellular and molecular ‘surrogates’ expected to correlate with cancer risk, e.g., the extent of DNA damage and repair, oxidative stress, and genomic instability (15-17). Likewise, at the other end of the spectrum, conclusions as to how cells
advance through the steps leading to cancer presentation are inferred from epidemiologic data on cancer incidence (18) in connection with cell culture and animal natural history models of tumor development (19, 20). Amidst the various linkages thus made, one presumption that has been consistently applied from the multistage mutational paradigm is that a cancer cell, once created, will eventually manifest as a cancer case (6, 21-23). By this standard, once a cell transforms to become a cancer cell, its progression to manifest disease is inevitable and the risk of eventual cancer incidence certain (left curve, Figure 2).

Countering this notion, a vast number of direct observations at the level of tumor progression are showing interactions of cancer cells with themselves and with cells of the host, may not only promote, but also slow, stop or even reverse a carcinogenesis course (24-30). One is confronted with the realization that cancer risk determination from exposures to carcinogenic agents not only requires a multi-scale effort to link the various levels at which data are available (from molecular to cellular to tissue to epidemiologic), but that the risk itself is influenced at multiple scales, having substantial population- and tissue-level components (Figure 1; Figure 2, right curve). Well established are the supportive and/or suppressive roles of tumor-associated elements, e.g. stroma, vasculature and the immune response in modulating cancer progression (31-33). Even after cancer cells arise, there are a number of population-level processes that need to be engaged to permit advancement through progression, including immune activity (34-37), invasive activity (38, 39), tumor angiogenesis (40, 41), and facilitation of stem cell proliferation (42, 43), including the need for recruited helper cells for stem cell expansion (44).

The failure of the cancer cell aggregate to mobilize host cooperation, or to evade host suppression, may cause the cancer to halt its progression, or even regress (45). In this regard, those key host processes that require modification to permit cancer progression amount to “bottlenecks”, the surmounting of which is essential for cancer incidence. The failure of cancer cells to recruit blood vessels and to overcome host immune surveillance, constitute two common progression-level bottlenecks, either of which could hold a tumor in a dormant state for an extended period (46-48). Recently, we have shown in murine studies that even host age itself (49) can modulate tumor progression, with the greatest tumor growth curtailment coming from the oldest hosts. Yet, some of the most compelling data on progression-phase limitations to tumor growth come from human data from autopsies of
individuals dying of non-cancer causes. Once thought anomalous, ‘dormant’ cancers were shown to be more the rule than the exception, with the discovery that most ‘cancer-free’ individuals harbor small, indolent tumor lesions that will not progress to manifest disease (25, 27, 50, 51). These and other findings have raised the concern that as our ability to screen for cancer improves, so will our tendency to treat lesions that pose no threat (51, 52). Reinforcing this point, although lethal lung cancer is 10 times more common in smokers, spiral CT screening is revealing non-smokers have about the same number of lung lesions as smokers (53, 54). Suggested is a strong role for disturbance of the local tumor-host interaction in the advancement of dormant lung lesions towards frank disease. The reach of host-derived progression modifiers may even extend to the provocation of transformation itself. A seminal study by Bhowmick et al. (28) confirmed the ability of host fibroblasts, in which the TGF-beta-type-II-receptor was inactivated, to elicit the transformation of adjacent normal epithelia. Carcinoma-associated fibroblasts have also been observed to convert genetically abnormal (initiated) but non-tumorigenic epithelia to carcinomas (29). Together, these observations appear to support an augmented version of the mutational paradigm; one that includes disruptions to homeostatic tissue-level signaling as potential initiators of early cell-level carcinogenesis, with DNA lesions being at times a consequential action. Lending credence to this notion, the immune response represents a tissue-level interaction that has been shown to influence both carcinogenesis progression and initiation. This can be accomplished by the secretion of certain cytokines and growth factors, which encourage proliferation of epithelia while at the same time generating DNA-damaging reactive oxygen species as part of their general infection-fighting function, further potentiating tumor development as per the classic mutational paradigm (55, 56). With regard to the known increased cancer incidence with aging, one may be seeing a gradual compromise of natural immune homeostatic surveillance that rids the body of damaged cells potentiated to become cancerous (57). At the same time, there is evidence that in later stages of cancer growth, the immune system and inflammatory response play a promoting role through a number of mechanisms, including dendritic cell depletion (58), recruitment of metastasis-promoting mesenchymal stem cells to the tumor site (59), induced accumulation of immunosuppressive myeloid-derived suppressor cells (60), the directed maturation of T cells from an anti-tumor Th1 phenotype towards a tumor-associated Th2 phenotype (61, 62); or generally through a process called immunoediting (63, 64). Reductions of these tumor-promoting aspects of immunity in older individuals may contribute to slow the progression of existing cancers. Taken together, these results help to explain how
immune compromise with age can facilitate cancer incidence while inhibiting tumor progression. The dichotomy of early tumor suppression and late tumor promotion is also played out by TGF-beta. One the one hand, TGF-beta can inhibit the initial growth of epithelial populations (65) and prevent tumor formation (66), while on the other, higher levels of TGF-beta in blood (67) and the tumor-microenvironment (68), along with age-dependent reduction in host expression of TGF-beta1 (Beheshti et al., unpublished data), have been associated with accelerated/pro-metastatic and reduced progression of existing tumors, respectively.

More generally, constraints imposed on cancer stem cell expansion by non-stem cancer cells may also retard tumor growth (42). In this setting, non-stem cancer cell death is paradoxically seen to speed up overall tumor growth, while the presence of non-stem cancer cells slows overall growth. The potentially systemic reach of mediation of tumor progression is supported by the finding that methionine metabolism, done primarily by the liver, correlates strongly with prostate cancer recurrence after radical prostatectomy (69). While a direct connection is not yet clear, that a fundamental metabolic pathway should be so intertwined with cancer progression offers highly suggestive evidence that cancer progression is a system-level process having system-level controls.

The emerging realization is that cancer development entails not just negotiating processes en route to producing a cancer cell population, but involves larger-scale population-, tissue- and organism-scale influences that accelerate or suppress progression of the nascent cancer population. This realization has major ramifications for carcinogenesis risk estimation and the development of countermeasures. In particular, it contradicts the common presumption in risk analysis of a direct coupling between the molecular steps in cellular oncogenesis leading up to transformation, and the final epidemiologic endpoint of cancer incidence (left curve, Figure 2). The critical roles of post-transformational cell-cell and cell-host interactions as “tissue-scale checkpoints” – potential barriers to tumor advancement – have begun to be appreciated in principle, but remain to be incorporated in quantitative modeling constructs as pivotal risk modifiers (right curve, Figure 2). Of specific concern here is that, since the effects of any progression-level bottlenecks on tumor advancement are already folded into the epidemiological data, risk models that ignore these inputs by assuming cancer incidence is inevitable once cancer cells are created, cannot correctly back-infer the cancer’s ‘natural history’ (i.e., the mutational, population-level, and host-interactive steps leading up to cancer incidence). In such analytic models, any reduction in tumor incidence due to such bottlenecks...
to tumor advancement will necessarily be taken into account through an inappropriate inflation of the estimated number of pre-transformation mutational events necessary to create the first cancer cell.

To avoid this disparity, it is clear there is a need to better interface biologically-based mechanistic carcinogenesis models with both natural history models and their epidemiologic cancer incidence endpoints. Meanwhile, research into the role of post-transformational mechanisms in cancer development is proceeding at a rapid pace. With it, a broadened picture of tumor progression is emerging that awaits integration into existing carcinogenesis models to provide improved risk assessments. In particular, the recognition that dormant microscopic tumors are highly prevalent in the population is poised to add a clear and measurable progression-level component to the multistage mutational theory of carcinogenesis. Knowledge that inter-tissue interactions among cancer, stromal (70), immune (61) and endothelial (41, 71) cells can radically alter the development of existing cancer cell populations is revising our understanding of cancer staging. It is also extending our understanding of cancer cell genetics itself by showing how accommodation of microenvironmental cues may exert overriding control over the expression of a pre-existing aggressive potential (72). This contrasts with tumor-cell-centric thinking on this point, which has posited such potential is only acquired gradually through mutation over the course of cancer development (72). Rounding out this picture, the physical and molecular determinants of altered stromal signaling (28), a permissive angiogenic state (41), environmentally-modified cancer stem cell kinetics (42, 43), the role of recruited cells in aiding cancer stem cells to drive tumor development (44), and aspects of the immune response that facilitate tumor progression (56, 57) are being elucidated.

Looking ahead, as the traditional considerations of intra-cellular, molecular-scale hallmarks of carcinogenesis are joined through modeling with consideration of the equally influential roles played by multiple tissue-scale bottlenecks – endpoints that tend also to be macroscopic and thus potentially easier to track – the relative roles of these intra-cellular molecular events will be more properly contextualized. Risk projections stemming from such multi-scale modeling efforts would not only better inform policy, but help in the identification of additional therapeutic control points in cancer development.
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Bibliography


Figure Captions:

**Figure 1. Carcinogenesis timeline.** Shown is the standard natural history timeline for cancer development. By this timeline, a chance DNA-altering event (e.g., a point mutation, deletion, translocation, copy number change, or heritable epigenetic alteration) "initiates" a cell, i.e., alters its growth characteristics and predisposes it for later carcinogenic conversion. In a phase called "promotion", the progeny of this initiated cell go on to display modified growth characteristics and altered propensities to undergo further spontaneous or induced genetic change. This cycle of DNA alteration and clonal expansion is revisited a number of times. Ultimately, a single cell in the clone undergoes a "transformation" event, becoming a frank cancer cell, which expands within the host tissues to become overt disease. As shown, what is classically understood to begin as a cell-level series of events graduates to include population-level effects shortly after initiation, then comes to include tissue-level influences as the nascent tumor grows and acquires larger-scale properties, e.g. invasiveness and metastatic potential, through interaction with the host. The periods of applicability are shown as solid red, green and blue lines, respectively. It is becoming increasingly clear, however, that each of these scales of influence may extend across the carcinogenesis spectrum, as indicated by the dashed extensions of these lines.

**Figure 2. Risk implications comparing the standard carcinogenesis risk paradigm to that accounting for progression-level effects.** The cancer risk of relevance for policymaking and quantitative model building is that of disease presentation. By the time cancer presents, a variety of expansion-limiting progression-phase bottlenecks have been successfully traversed by the cancer cell population, any one of which could have blocked the carcinogenesis process. Beyond the oncogenic mutations that must be acquired for a cell to transform, population-level acquisitions, including angiogenic and invasive capacity, immune escape, and stromal permissiveness, must be in place for transformed cells to advance to become symptomatic disease (waving curve, extending left to right). Interruption of tumor progression through any one of these bottlenecks could be decisive in whether overt disease ever arises. Notwithstanding, standard risk modeling approaches typically assume cancer cells, once created, progress inevitably to disease presentation, and that cancer incidence rates are therefore a direct reflection of the numbers and natures of the genetic alterations required to produce a cancer cell. This depiction of the carcinogenesis process has the effect
of assigning the entire risk of disease presentation to that of transformation of an initiated cell to a cancer cell (leftmost curve, steeply rising), an event that autopsy evidence refutes, suggesting transformation is but a first step towards manifest disease (portion of waving curve that lies below the leftmost curve). An alternative risk model that includes post-transformation determinants of carcinogenesis from the outset would provide not only more realistic risk estimates, but also an improved understanding of both the pre-tumor-cell and post-tumor-cell contributions to the overall carcinogenesis process.
Figure 1

normal tissue

initiation → promotion → transformation → progression → manifest cancer

Cell level

Population level

Tissue level
Components Increasing Risk
- Normal fibroblast
- Anti-angiogenesis factor
- Anti-tumor immune modulator
- Anti-tumor immune cell
- Extended Progression-Based Paradigm

Components Decreasing Risk
- Tumor cell
- Pro-tumor immune modulator
- Pro-angiogenesis factor
- Pro-tumor immune cell
- Stromal factor
- Tumor-associated fibroblast

Clinical presentation
Figure 2
Beyond the Cancer Cell: Progression-Level Determinants Highlight the Multi-Scale Nature of Carcinogenesis Risk

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