Molecular Insights into Cancer Invasion: Strategies for Prevention and Intervention

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Abstract

The diagnosis and treatment of solid tumors usually begins at a late stage when most patients already have occult or overt metastasis. Many years of cancer progression precede diagnosis of most solid tumors. Novel noncytotoxic therapeutics may be specially suited for administration during this interval. An important window of intervention can be defined as the period during which transition from a hyperproliferative state to acquisition of the capacity for invasion and metastasis occurs. Investigation of the molecular basis of invasion is uncovering strategies for delaying progression of preinvasive carcinoma and treatment of primary tumors and established metastasis. Although tumor cell invasion might not be rate limiting for the growth of metastasis, anti-invasive agents can block tumor angiogenesis and thereby indirectly block metastasis growth. Two classes of molecular anti-invasion targets exist: (a) cell surface and extracellular proteins, which mediate sensing, adhesion, and proteolysis; and (b) signal transduction pathways, which regulate invasion, angiogenesis, and proliferation. Both categories of targets yield treatment approaches that are now being tested in the clinic. Metalloproteinase inhibitors, such as BB94, are based on the recognition that metalloproteinases play a necessary role in invasion and angiogenesis. The orally active signal transduction inhibitor carboxamidotriazole modulates non-voltage-gated calcium influx-regulated signal pathways and reversibly inhibits tumor invasion, growth, and angiogenesis. Blockade of invasion, angiogenesis, or cellular signal pathways is likely to generate a cytostatic, rather than a cytotoxic effect. Cytostatic therapy constitutes an alternative paradigm for clinical translation that may complement conventional cytotoxic therapy. For patients with newly diagnosed solid tumors, long-term cytostatic therapy could potentially create a state of metastasis dormancy or delay the time to overt relapse following cytotoxic agent-induced remission. Clinical toxicity and pharmacology using oral cytostatic agents in phase I trials and in adjuvant settings will provide an important foundation for the translation of this approach to the preinvasive carcinoma period.

Introduction

The overt manifestation and initial presentation of cancer usually occur at a late stage in the disease process when the capacity for invasion has already been unleashed. By the time of diagnosis, a high proportion of patients have occult or clinically detectable metastasis. The capacity of conventional cytotoxic approaches to succeed in the face of this advanced, accelerating disease has unfortunately been limited (1, 2). In contrast to the short time between disease presentation and established metastasis, the chemoprevention period (Fig. 1) may extend back 10 years or more (3–6). For breast cancer, the period of transition from hyperproliferative but noninvasive disease (3, 5, 7, 8) to invasive cancer is estimated to average 6 years (3, 5).

We have recently obtained direct genetic evidence for the previously unproved assumption that in situ breast cancer is a clonal precursor of invasive carcinoma (7). Carcinoma in situ of the breast can now be viewed as a field disease spanning the clonal expansion of hyperproliferating cells that permeate the ductal system (3, 5, 7, 8). The neoplasm may remain confined to the intraductal space until additional genetic progression events occur that instill the capacity for invasion. It is assumed that dissemination is impossible until this step has occurred. The time period after the invasive carcinoma grows to reach the minimum threshold size of detection (0.25 cm in diameter), but prior to establishment of the first metastasis, can be less than 1 year (5, 8). Thus, incorporating the transition of preinvasive carcinoma to invasion provides a much larger window for screening and intervention compared to the conventional goal which focuses on established invasive carcinomas.

The critical pathological turning point that defines this new prevention target is the initiation of local invasion leading to the dissemination of tumor cells. Tumor-induced neovascularization occurs in parallel with the transition to invasion and provides a vascular entry portal for dissemination which may precede evident primary tumor outgrowth by many years (9–11) (Fig. 1). Circulating tumor cells can be detected in patients who never succumb to metastasis (9). This clinical observation is in keeping with experimental studies indicating that metastasis initiation from circulating tumor cells is a low probability event. Less than 0.05% of circulating tumor cells are successful (10, 12). The first metastasis is established only after a sufficiently large number of tumor cells containing the necessary genotypic and phenotypic changes have entered the tumor venous and lymphatic drainage (12, 13).

Agents that either block angiogenesis or retard invasion can offer an alternative approach for arresting neoplastic progression at the stage of hyperproliferative noninvasive cancer. Using the example of breast cancer, in situ carcinoma remains unvascularized, is strictly limited in size, and is separated from the stromal lymphatics by a basement membrane barrier (14). Thus, this stage of cancer may be effectively benign. Furthermore, since the incidence of in situ carcinoma decreases in later ages (5, 8), we can hypothesize that this multifocal ductal lesion may ultimately undergo regression if progression to invasive cancer does not occur. Preventing the malignant evolution of incipient in situ carcinoma may be just as clinically effective as blocking neoplastic progression at an earlier stage.

Molecular Checkpoints That Regulate Invasion

The molecular characterization of invasion and angiogenesis has led to the identification of two categories of checkpoints that constitute intervention targets. The first category encompasses cell surface and secreted proteins. The second category is defined as regulatory proteins and pathways inside the cell. Positive and negative elements fall under the first category include cell surface and secreted proteins such as adhesion receptors, degradative enzymes and their inhibitors, and motility-stimulating cytokines (17–22). The second checkpoint category includes regulatory proteins and pathways such as calcium-mediated signaling, G-protein activation, and tyrosine phosphorylation events (23–26). Examination of the structure and function of checkpoint molecules provides the basis for development of agents that can potentially block tumor invasion or growth.

Cancer Invasion: Spatial and Temporal Regulation at the Cellular Level

Invasion is the active translocation of neoplastic cells across tissue boundaries and through host cellular and extracellular matrix barriers.
Invasion is not simply due to growth pressure but involves additional genetic deregulation over and above those molecular events that cause uncontrolled proliferation. Expression of the malignant phenotype is probably not caused by one single gene or protein. Instead, invasion results from an imbalance and deregulation of what are normally stimulatory and inhibitory events. This is especially important in that all of the components of invasion play important roles in tightly controlled normal physiological events. At the biochemical level, the mechanism of invasion used by tumor cells, may parallel, or be similar to, that used by nonmalignant cells, which traverse tissue boundaries under normal physiological conditions (16).

Examples of physiological invasion are smooth muscle cell migration from the media to the intima (27), angiogenesis (11, 28), embryogenesis and morphogenesis (29), nerve growth cone extension and homing (30), and trophoblast implantation (31). In contrast to malignant invasion, physiological invasion is tightly regulated and ceases when the stimulus is removed. Angiogenesis will cease when the source of the angiogenic factor is removed (11). Neurite migration and invasion is arrested when the neurites sense destination arrival cues (30). Differentiation of trophoblasts terminates their physiological invasion (31). In counterdistinction, invading tumor cells appear to have lost the control mechanisms which prevent normal cells from invading neighboring tissue at inappropriate times and places (16, 32).

If one assumes that the malignant tumor cell is inappropriately expressing a pre-existing normal cell program of physiological invasion, then the fundamental difference between normal and malignant cells is regulation. The difference must lie in the proteins that start, stop, or maintain the invasion program at times and places that are inappropriate for nonmalignant cells (12, 16, 32). A major goal, therefore, is to understand what signals and signal transduction pathways are perpetually activated or unrestrained in malignant invasion compared to physiological invasion.

**Cellular Invasion: Action and Reaction at the Pseudopod**

Regulation of molecular events necessary for invasion, malignant or physiological, requires spatial and temporal coordination and cyclic on-off processes at the level of the individual cell. Cellular invasion is dependent on the coordinated activity of a series of interacting proteins extending from inside the cell, through the plasma membrane, to the cell surface and pericellular microenvironment (Fig. 2). For cells migrating within a three dimensional matrix, such as penetrating a basement membrane, protrusion of a cylindrical pseudopod is the first step, prior to translocation of the whole cell body (33, 34). Pseudopodial cell fragments, devoid of nuclei, retain pertinent sensing and directional locomotion capacity (33, 34), proving that these organs of translocation have all the sensory and motor equipment necessary for crawling.

A number of observations support the central role of cytoskeleton-driven pseudopodia as organs of motility and invasion. Pseudopodia protrude at the point of surface stimulation by appropriate ligands which cause migration (33–35), and they appear to aggregate or concentrate cell surface degradative enzymes and adhesion receptors (35). Pseudopods have an internal supporting actin structure that causes the pseudopod to extend into free space at the confined area of ligand binding (34, 36).

A series of proteins on the surface of the pseudopod coordinate sensing, protrusion, burrowing, and traction. Proteinases at the pseudopod tip may locally disrupt the extracellular matrix and permit forward extention. However, the balance must switch from proteolysis to adhesion in order for the advancing pseudopod to grip the matrix and pull the cell forward. General unregulated proteolysis alone cannot be responsible for the entire invasion cascade because it would remove the substratum necessary for proper traction. The cell would, in effect, dig itself into a hole and become immobilized. Consequently, to achieve forward locomotion, the invading cell couples local proteolysis (16) with coordinated and temporally limited attachment and detachment (37). At the leading edge of the cell, proteinases bound to activator proteins and receptors may be colocalized on the advancing tip of the pseudopodia. When the cell moves into the zone of lysis, adhesion is required and proteolysis must be shut down (37, 38). At the rear of the cell, dissociation from adjacent cells, and detachment (18, 19) from previous attachment sites are necessary to release the cell (Fig. 2).

**Regulation of Invasion Extends to the Cell-Matrix Interface**

The basement membrane and interstitial stroma play an important regulatory role in malignant as well as physiological invasion. Basement membranes constitute barriers that confine the movement of cells to specific tissue compartments. The basement membrane may...
also be a storage depot for latent proteinases and cytokines including angiogenesis factors, which can be activated or released by the invading cell pseudopodia. Abnormal, dysfunctional, or fragmented basement membranes accompany physiological as well as malignant invasion. Basement membranes may be defective due to altered assembly or disruption by augmented local activity of matrix-degrading proteinases. It is now well established that one way the integrity of the basement membrane can be regulated is by the balance between metalloproteinases and metalloproteinase inhibitors (14, 20, 29). Under this hypothesis, agents or treatments that maintain the integrity of the epithelial basement membrane may be an alternative approach to retard invasion or angiogenesis. For example, carcinoma in situ lesions of the breast, if induced to remain confined within the ductal epithelial basement membrane, may remain in a dormant state, unable to become vascularized, and unable to disseminate.

Anti-invasion Defines a New Category of Cancer Chemoprevention

Arresting or retarding molecular events involved in tumor invasion and angiogenesis defines a new category of chemoprevention agents. Overlapping regulation of growth and invasion occurs at the cellular and extracellular level. Consequently, anti-invasion agents may also be cytostatic (23, 26). Chemoprevention has traditionally been defined as the use of one or several chemical agents to prevent the occurrence of cancer (4, 6, 23). Chemoprevention agents have been placed into two major categories, blocking agents and suppressing agents (4, 6). Blocking agents have a barrier function to prevent cancer-producing substances from reaching or reacting with cellular target sites. Given the time at which initiation and promotion events occur in stem cells, blocking agents, in order to be effective, may need to be administered at an early age much before adulthood. According to the report of Wattenberg (6), suppressing agents act after exposure to cancer-producing compounds and prevent the evolution of the neoplastic process in cells that otherwise would become malignant.

In theory, some suppressing agents stimulate differentiation, while others reverse the consequences of activated oncogenes and suppressor genes. For example, retinoids and vitamin D compounds have the capacity to induce differentiation, and H-ras oncogene action can be blocked by monoterpenes or isoprenylation inhibitors (25). Another group of suppressing agents selectively inhibits proliferation of potentially malignant cells (6). Under this group, hormone antagonists and hormone metabolism inhibitors are suppressing agents that retard cell proliferation.

The mission previously posed for classical chemoprevention agents did not encompass the full spectrum of malignant progression. The transition period from the hyperproliferative state to the emergence of tumor cells with lethal capacity can occupy a major fraction of the chemoprevention window. This affords a potentially safer point of therapeutic entry in late adulthood compared to high risk treatment at earlier ages, which theoretically would be required to block initiation of transformation.

Matrix Metalloproteinases: From Correlation to Viable Therapeutic Target

A positive correlation between tumor aggressiveness and protease levels has been documented for all four classes of proteases including serine, aspartyl, cysteinyl, and metal atom dependent (20, 38–47). In addition to proteolytic activity, augmented heparanase activity has also been associated with malignancy (45). All of these classes of enzymes may be equally important at one or more stages of invasion. Nevertheless, progress made in the field of metalloproteinases serves as an illustrative example of how specific inhibitors can be used to simultaneously test the proteinase hypothesis and explore therapeutic strategies.

Significant evidence has accumulated to directly implicate members of the gene family of matrix metalloproteinases in tumor invasion and metastasis formation. These enzymes, each of which is secreted as a proenzyme that requires activation, are divided into three general subclasses (48–50): (a) interstitial collagenase; (b) stromelysins; and (c) gelatinases (type IV collagenases). Interstitial collagenase degrades type I collagen, as well as the fibrillar collagens II, III, and X. The stromelysins degrade proteoglycan core protein, laminin, fibronectin, gelatin, and the nonhelical portions of basement membrane collagens. Stromelysin 3 is associated with human breast cancer stromal cells, but its substrate specificity has yet to be defined. Matrilysin, but not stromelysin 1 or stromelysin 2, is prominent in human gastric and colonic carcinomas. In epidermoid carcinomas of the head and neck, stromelysin 2 as well as interstitial collagenase mRNAs were detected in invasive cancer cells and associated stromal cells, whereas adjacent normal tissues were negative. Furthermore, stromelysin 2 mRNA was localized in tumor cells arranged along disrupted basement membranes. These studies suggest that there may be organ or cell type specificity associated with the up-regulation of proteolytic activity during malignant conversion.

The third group of enzymes of the MMPs gene family are the type IV collagenases. The M, 72,000 and M, 92,000 enzymes (MMP-2, MMP-9) were originally named for their ability to degrade pepsinized, triple-helical type IV collagen. In addition, they possess potent gelatinolytic activity and can degrade collagen types V, VII, IX, and XI; fibronectin; and elastin. The two gelatinase enzymes arise from separate mRNA transcripts on separate genes. They are distinct from other members of the matrix metalloproteinases in that they possess a unique region immediately adjacent to the metal-binding domain that is homologous to the gelatin-binding domain of fibronectin. Gelatinases A and B differ from other metalloproteinases by their ability to interact, as latent proenzymes, with the endogenous TIMPs (TIMP-1 and TIMP-2). The gelatinase A proenzyme forms a complex with TIMP-2, and the M, 92,000 gelatinase B proenzyme binds with TIMP-1 (49–55). TIMPs are not known to bind other latent matrix metalloproteinases but will inhibit all matrix metalloproteinases once these enzymes are activated. Thus, TIMP-2 preferentially recognizes the latent form of gelatinase A.

Correlative evidence for the involvement of gelatinase A and gelatinase B in the invasive phenotype is abundant (56–59). Induction of the malignant phenotype using the H-ras oncogene has been shown to enhance expression of gelatinases A and B (56). Most invasive colonic, gastric, ovarian, and thyroid adenocarcinomas or the desmoplastic stroma of breast lesions have been shown to be immunoreactive for gelatinase A, whereas benign proliferative disorders of the breast and colon and normal colorectal and gastric mucosa and benign ovarian cysts show decreased or negative staining for this enzyme (57, 58). However, gelatinase A was also detected in benign disorders in which the tissue was undergoing remodeling and repair such as in inflammation, fibrosis, and distortion of normal follicles. In contrast, normal thyroid, goiter, and Graves disease tissue showed little or no immunoreactivity.

Connective tissue destruction by metalloproteinases is largely irreversible. Consequently, selective inhibitors of these enzymes offer important tools with which to study the role of MMPs in physiological and malignant invasion (59–64). MMP inhibitors also constitute viable strategies for disease intervention. Structure-function information about MMP enzyme domains, activation mechanisms, and the
interaction with TIMPs has led to a number of successful inhibitor strategies. One approach is to use TIMPs or TIMP fragments directly as selective inhibitors of MMP activation or activity (46–53, 59–63). A second strategy is peptide inhibitors, which mimic theaminomethyl MMP motif that maintains the latent enzyme state (64–67). A third example is synthetic compounds, which compete for the substrate or bind to the active site (64, 68–70).

In keeping with the concept of parallel mechanisms for physiological and malignant invasion, all three types of metalloproteinase inhibitors have been shown to inhibit physiological invasion models. TIMP-1 and TIMP-2 block trophoblast invasion. TIMP-1, TIMP-2, and synthetic substrate inhibitors inhibit angiogenesis in vivo (63, 64, 70). Gelatinase A peptide inhibitors and synthetic substrate inhibitors block neurite outgrowth (69). Synthetic substrate inhibitors also have been shown to prevent smooth muscle cell migration from the vascular intima to the media following arterial balloon injury (27). Collectively, these data support a role for collagenolytic activity in at least two functional processes contributing to metastasis. That is, collagenases are involved in tumor cell invasion as well as neovascularization, upon which solid tumor growth is dependent. These are important considerations in the design of proteolytic inhibitors for potential use as therapeutic agents.

Native or recombinant TIMP-1 has been shown to inhibit in vitro invasion of human amniotic membranes and in vivo metastasis in animal models (47). Furthermore, transfection of antisense TIMP-1 RNA into mouse 3T3 cells, which down-regulates TIMP-1 expression, enhances their ability to invade human amniotic membranes and to form metastatic tumors in athymic mice (60). TIMP-2 is a M, 21,000, nonglycosylated protein that shows 37% identity and 65.6% overall homology to TIMP-1, yet the two proteins are immunologically distinct and are encoded by genes on separate chromosomes (51–53). Exogenous addition of TIMP-2 has also been shown to successfully inhibit in vitro tumor cell invasion of extracellular matrices (61, 62). Overexpression of TIMP-2 in invasive and metastatic ras-transformed rat embryo fibroblasts suppressed the formation of lung metastases following i.v. injection in nude mice (62).

In vitro studies of the mechanism of activation of MMPs enzymes has yielded new insights into the molecular basis of proenzyme latency. Activation is produced by the loss of an 80-residue NH2-terminal domain containing the sequence PRGXPDV, which is highly conserved among family members (65–67). The latency of the metalloproteinases appears to be maintained by a specific metal atom-sulfhydryl side chain interaction with the cystinyl residue. This autoinhibitory consensus peptide has been used to develop a family of inhibitor peptides, some of which have a 50% inhibitory concentration of 3 μM. These inhibitors require the single cysteine residue to be effective in blocking the enzyme or halting invasion (65–67). The cysteinylic-containing peptide prevented more than 80% of the invasion of reconstituted extracellular matrix by human fibrosarcoma, breast carcinoma, or melanoma cells. Although effective and specific, the clinical utility of these cysteineyl peptide inhibitors is currently limited until orally active derivatives are available.

The largest current group of synthetic metalloproteinase inhibitors consists of the collagen substrate analogues. All are less than six amino acids long (64). Up to 3 of the amino acids are placed on 1 side of the collagen scissile bond, and the peptide is linked to a Zn2+ binding moiety. This design targets the Zn2+ atom coordinated within the active site of all MMP molecules. A variety of Zn2+-chelating moieties have been used, among them hydroxamic acid, thiol, and carboxyalkyl groups. It is theorized that these compounds act by binding to the active site of the metalloproteinase. An example compound is BB94 (Batimastat) (64, 68, 71). BB94 is active in vitro with a 50% inhibitory concentration of 20 nM for stromelysin and 4 nM for gelatinase A. Administration of BB94 i.p. to athymic nu/nu mice bearing fragments of human colon carcinoma inhibited the growth of the primary tumor, caused a marked reduction in the incidence of tumor invasion of local tissue, and reduced the incidence of spontaneous metastasis (68, 71). In a separate study, BB94 significantly delayed the growth of transplanted B16-BL6 primary melanoma and reduced the size of established metastasis. As a test of the theory that metalloproteinases may play a role in peritoneal invasion of ovarian cancer (71), BB94 was shown to decrease tumor burden and prolong survival of mice bearing human ovarian carcinoma xenografts. The efficacy seen in these preclinical studies supports the basic hypothesis that metalloproteinases are important effectors of invasion and metastasis. The suppression of tumor growth may be indirectly linked to an effect on angiogenesis (16, 63, 70). Furthermore, a component of tumor expansion may be dependent on the invasion of adjacent tissue. This strong rationale, combined with promising preclinical data, has been the basis for ongoing clinical trials exploring the use of BB94 in patients with advanced cancer.

**Calcium Homeostasis Modulators Block Invasion, Growth, and Angiogenesis**

The second molecular checkpoint category contains the regulatory proteins and signaling pathways of the cell. Cellular communication circuits can be modulated to produce a positive or negative downstream action depending on the location and type of intervention. Examples are differentiating agents, enzyme modulators, and direct signal transduction effectors (72–77). Targeting signal transduction pathways provides a novel approach to down-regulate the invasive process, and one that may potentially allow earlier intervention during the invasion prevention window described above. The accumulation of molecular events during cancer progression changes the signaling homeostasis of cancer cells through mutation, altered expression, and the development of autocrine loops upon which the cells depend. The goal of signal transduction therapy is to suppress hyperactive or aberrantly active signaling pathways of malignant proliferation and invasion. This class of agents may therefore be expected to be cytostatic rather than cytotoxic. The redundancy in signaling pathways in nonmalignant cells can theoretically create checks and balances that would protect against normal cell toxicity during continuous exposure to signal modulatory drugs (23, 26, 78–84). In contrast, classical chemotherapy agents, which can inhibit invasion and metastasis by their cytotoxic activity, target DNA synthesis, repair, and transcriptional events. The frequent global toxicity patterns of cytotoxic agents is a consequence, in part, of the central action at the DNA level. There are several signaling pathways to which drug development may be directed. These include nuclear events, such as transcription; however, drug delivery may be a problem. It may be difficult to selectively target nuclear events either in directing the drug to its site of action or to direct it to the specific event in question. For example, the AP-1 transcription factor is important in the process of invasion and metastasis by its activation of transcription of stromelysin and gelatinase B (39, 40, 77), as well as being important in signaling events of proliferation. Targeting of AP-1 as a mechanism by which to intervene therapeutically will require not only delivering the agent to AP-1 but also creating a level of selectivity. Since signals originating peripherally in the cell may route through several subsequent pathways, peripheral signal targets may allow for more selectivity by allowing normal checks and balances within the signaling homeostasis of the cell to intervene (23, 24, 26, 78–80, 83–87). Signaling events in the cytoplasm or at the nucleus, such as post-translational modifications and transmembrane signaling, may provide more accessible checkpoints. Transmembrane or cytoplasmic signaling events
proposed for drug intervention have included inhibition of posttranslational modifications such as prenylation (25, 88), modulation of protein kinase activity (76), and alteration in ion homeostasis, such as calcium influx (23, 26, 75, 81).

Altered signaling pathways have been tied to the process of invasion and metastasis. A prime example is the introduction of activated H-ras into primary rat embryo fibroblast cells. This transfection (89) resulted in development of a malignant and invasive phenotype, characterized by production of gelatinase B and marked pulmonary colonization after tail vein inoculation of transfected cells. The invasive phenotype could be differentiated from the tumorigenic phenotype by further transfection with adenovirus Eia. Similar effects have been observed with expression of other oncogenes, such as Her-2/neu (90). When Eia was transfected into neu-transformed NIH-3T3 cells, it abrogated the metastasis-associated properties imparted by neu and also its transforming effects (90). Re-expression of neu resulted in return of transforming potential without metastatic capabilities. These studies reinforce the overlap between oncogene-induced tumor development and dissemination but underscore that distinct signals must be in place for the functional separation of transforming activity and metastasis to occur. The invasive phenotype may be regulated by signal transduction pathways downstream from activated oncogenes.

Intracellular calcium is an important regulator of subsets of the three transmembrane signaling categories: (a) ion fluxes; (b) phosphorylation events; and (c) guanine nucleotide (G)-binding protein-mediated second messenger production (26, 72, 85–87, 91). Intracellular calcium may be mobilized from within the cell from endoplasmic reticulum stores released in response to inositol trisphosphate, or it may enter the cell through voltage-gated or non-voltage-gated calcium channels (24, 78, 79, 83–87, 91). Most epithelial cells, parental cells of carcinomas, are electrically neutral and do not have voltage-gated calcium channels as their primary method of calcium entry. This defines non-voltage-gated calcium influx as the primary regulator of cellular signaling pathways. We have identified an inhibitor of non-voltage-gated calcium pathways, CAI, which we have shown to inhibit tumor cell proliferation, invasion, and angiogenesis both in vitro and in vivo (26, 78–84). The experimental efficacy of CAI suggests that this mode of intervention of the signaling of invasion may be a viable therapeutic option and will be discussed as an example of this therapeutic checkpoint target of invasion.

Early studies of CAI demonstrated that it inhibited non-voltage-gated calcium influx in response to several agonists (83, 84) in the concentration range of 1–10 μM. The CAI-sensitive calcium influx events have been linked to downstream signaling pathways including phospholipase A2, calcium-sensitive phospholipase Cγ, and protein tyrosine kinase activity (81, 83, 84).2 Modulation of these signaling pathways by cellular preincubation with this concentration range of CAI resulted in inhibition of the component parts of invasion. CAI exposure inhibited tumor cell migration (78) in response to autocrine motility factor (22), a G protein-mediated event, and type IV collagen, shown to involve calcium mobilization (92); adhesion to tissue culture plastic and type IV collagen has also been demonstrated (78). Cell exposure to CAI inhibits production of gelatinase A by reduction in gene expression (80). Coupled with inhibition of proliferation of a wide variety of malignant human cell types in vitro (79, 81), CAI could thus inhibit both tumor growth and invasive potential of malignant cells.

Angiogenesis, which may be permissive for the progression from carcinoma in situ to invasive carcinoma, is a form of regulated or physiological invasion requiring the same components of adhesion, proteolysis, and migration as do malignant cells. Human endothelial cell incubation with CAI resulted in the same inhibition of invasion as was seen for malignant cells, i.e., reduction in adhesion and in migration in response to an array of extracellular matrix component proteins and production of gelatinase A (82). This inhibitory activity was confirmed in vivo and all effects, in vitro and in vivo, occurred in the same concentration range as the inhibition of malignant invasion and the inhibition of signaling pathways.

Preclinical studies of CAI in mice demonstrated oral bioavailability and the ability to attain targeted signal inhibitory plasma concentrations without marked toxicity. Daily administration of CAI to human xenograft-bearing mice resulted in reduction in total tumor burden, tumor incidence, and metastatic dissemination (79). A similar marked reduction in experimental metastases was observed with cellular pretreatment in vitro prior to tail vein inoculation or p.o. administration of CAI prior to or after tail vein inoculation with CAI-naïve cells (79). Only minor gross or histological toxicity has been observed with p.o. dosing of CAI in preclinical studies. A phase I clinical trial of CAI in patients with refractory tumors has demonstrated the ability to attain plasma concentrations in the range that inhibits signaling and invasion in vivo, 1–10 μM, and shows an acceptable pattern of toxicity and promising evidence of cytostatic clinical activity. Phase III trials are in the planning stage.

Cytostatic Therapy: A New Paradigm

Identification of the molecular basis of tumor dissemination is driving drug development to discover or synthesize inhibitors that block key pathways in this process. Many of the checkpoints involved in cellular invasion have been shown to regulate angiogenesis (16), suggesting a dual therapeutic role for these inhibitors. Drugs identified to date, including metalloproteinase inhibitors (64) and signaling inhibitors (23, 26), have been shown to be reversible inhibitors of both invasion and proliferation. In fact, the general class of signal transduction inhibitors is expected to produce a cytostatic rather than a cytotoxic action.

The current cytotoxic treatment paradigm is aimed at identifying and applying drugs that cause a measurable reduction in tumor mass. This class of chemotherapeutic agents also has broad toxicity, particularly for rapidly growing normal tissues. Moreover, the reduction in tumor bulk produced by cytotoxic chemotherapy is all too often associated with tumor regrowth (1, 2), followed by repeat cycles of chemotherapy and regrowth, ultimately leading to a lethal outcome. While effective in reducing tumor burden, classical chemotherapy has produced true cures in only a small percentage of patients with metastatic cancer.

The goal of the cytostatic paradigm of intervention is to hold metastatic dissemination at bay, slow or halt proliferation, and generate a period of disease stabilization (Fig. 3). For patients with established systemic metastasis, the goal of chronic treatment would be to place, and keep, the metastatic colonies in a state of dormancy. Over time it is possible that the high death rate of tumor cells, especially nonhematological malignancies, may overbalance the arrested growth yielding slow regression. Disease stabilization or slow regression as an end point is contrary to current standards of clinical investigation, which seek immediate tumor mass reductions. Consequently, under the paradigm of cytostasis, new end points of clinical efficacy will have to be developed, such as time to progression, quality of life, and survival. In addition, it will be important to develop new methods to monitor tumor cytostasis in the patient.

Because chronic treatment over long time periods is required for cytostatic agents, they must be preferably of a formulation that is readily administered and well tolerated, and therefore not compliance
limiting. If this criterion is met, the potential benefit for advanced disease is a reduction in the requirement for cytotoxic therapy, maintenance or reduction in the use of pain medication, stabilization or improvement in performance status, and ultimately delayed time to progression and extended survival. Indeed, improved quality of life alone may be an important criteria for efficacy of novel cytostatic agents (1). The trial agents discussed here and in the literature will constitute the first generation of invasion inhibitors and cytostatic agents. Toxicity and pharmacology in phase I trials and in adjuvant settings will provide an important foundation to move from the treatment arena to the invasion prevention period (Fig. 1).

Clinical cohorts for whom invasion prevention approaches might be applicable include patients with genetic risks of invasive cancers (4, 15). These cancers frequently begin the hyperproliferation period at early ages, and the clinical task of preventing tumor initiation and promotion in the setting of a defective germ line may prove difficult. Instead this population could be targeted for intervention during the progression period. The goal would be detection and management prior to invasion and metastasis. If successful, cytostatic therapy for patients genetically at high risk could provide an adjunct to surgical extirpation, perhaps minimizing the extent of surgery required for these patients with broad tissue/organ fields at risk. Information gleaned from these obligate cancer cohorts can then be used to design subsequent clinical investigations of cytostatic invasion inhibitor therapies.

Conclusion

The recognition that the process of malignant invasion is one of deregulated physiological invasion and is a dynamic process of temporal and spatially defined molecular events opens a new arena for therapeutic gain. Since physiological invasive processes are strictly delimited and under tight control, intervention directed against malignant invasion may be feasible, specific, and easily targeted. Cytostatic agents targeted to communication pathways that regulate proliferation or key proteins involved in invasion may be a way to introduce regulation into the molecularly rigid malignant cell and put brakes against its otherwise inexorable progression.

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