Reflections on the Spread of Metastasis to Cancer Prevention

Makoto Mark Taketo

Abstract
Most patients with a lethal cancer die of metastasis, the control of which deserves to be one of the most urgent missions of cancer treatment. For some subsets of colorectal cancer, metastasis is stimulated by chemokines or Notch signaling at early stages that coincide with the inflammatory phase of postoperative wound healing. This temporary phase may provide a unique opportunity for “metastasis prevention,” because some novel agents have been developed that target such chemokine receptors or Notch signaling.


Introduction
Based on progress in the molecular genetics and biology of cancer, our understanding of the mechanisms of carcinogenesis has advanced tremendously in the past few decades. Taking advantage of such information and of technical advances in imaging methods, the clinical diagnosis of cancer is becoming more accurate and precise at earlier stages of the disease. Development of molecular targeting therapeutics, such as tyrosine kinase inhibitors and humanized antibodies, has allowed much-improved prognosis and survival of cancer patients.

Unfortunately, however, cancer remains the major cause of disease deaths in most industrialized nations, and these cancer deaths result mainly from metastasis. For metastasis-free patients (both macroscopically and microscopically), surgical excisions of the primary tumors (and/or radiation therapy, when indicated) are quite successful in almost all cases. Thus, it is obvious that we can control cancer if we can control metastasis. In colon cancer, for example, the current 5-year survival (i.e., the cure rate) is approximately 80% to 90% for metastasis-free patients and only 10% to 20% for the overall group of patients with metastases (1). In the subset of metastatic cases who undergo liver-metastasis resections, about half are cured and the other half relapse with macroscopic metastases elsewhere in the liver (and/or in the lungs) that likely developed from disseminated tumor cells (micrometastases) that already existed at the time of surgery. Thus, the latter half patients could be cured if early-stage metastasis was blocked postoperatively.

Practically speaking, few current chemotherapeutics appear to be efficacious enough to cure metastatic disease that has already expanded to a macroscopic size. How can we improve this situation? Are there any means to prevent the metastatic growth of already disseminated cancer cells? In considering the possibility of “metastasis prevention,” that is, as adjuvant therapies to surgery (and/or radiation), I would like to start with the body’s reactions to surgery.

Inflammatory phase in wound healing
The whole rationale for surgical therapy is based on the body’s marvelous capacity for wound healing. Whether it is an inflamed appendix or cancer, surgeons can expose it to treat the patient simply because the human body recovers from the surgical injury through wound healing. This healing is a series of processes carried out by the combination of a variety of biochemical mediators secreted from a constellation of various inflammatory cells in a chronologically well-orchestrated manner (Fig. 1).

Following the initial bleeding of a surgical injury, blood coagulation and vasoconstriction take place in approximately 5 minutes. Then, vasodilation induced by histamine causes the approximately 2-week “inflammatory phase” of wound healing. Overlapping the inflammatory phase, the “proliferative phase” begins on about day 3 and lasts for several weeks, followed by the “remodeling phase” that continues for 6 to 12 months (2). Polymorphonuclear leukocytes initially recruited during the inflammatory phase are replaced by macrophages and lymphocytes, followed by recruitment of fibroblasts and endothelial cells in the proliferative phase, which involves fibroplasias and angiogenesis. These cells are attracted to wound sites by a succession of biochemical mediators released from the wounds and cause profound effects. Such mediators include a bunch of growth factors [including epidermal growth factor (EGF), transforming growth factor alpha (TGFα), TGFβ1-3, VEGF, fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), and keratinocyte growth factor (KGF)], interleukins (IL-1,-4, and -8), cytokines [granulocyte colony-stimulating factor (G-CSF), granulocyte macrophage CSF (GM-CSF)], chemokines, and lipid mediators such as prostanoids (3).
Although these factors play key roles in wound healing, it is a reasonable assumption that some of them can also stimulate the growth of metastases, as they can reach distant organs through the circulation. Indeed, accumulating evidence suggests that inflammatory and immune cells participate in cancer expansion and progression (4–7). For example, the role of tumor-associated macrophages in stimulating breast cancer progression has been demonstrated beautifully in the polyoma middle T-antigen–induced mouse model (8, 9). Likewise, chemokines secreted from cancer cells can fool other cells, including bone marrow–derived cells, by attracting them to the invading edge of the tumor and surrounding environment of micrometastases to facilitate metastasis (10, 11; Fig. 2).

Clinically, it has been recognized that metastatic tumors appear and expand faster after excision of primary tumors, as exemplified by the famous anecdote of Judah Folkman’s observation of this phenomenon and consequent proposal of the existence of angiotatin (12, 13). Moreover, some of the cellular oncogene products include growth factors and their receptors. Accordingly, it is conceivable that the surge of such biochemical mediators during postoperative wound healing can stimulate metastatic colonization of already disseminated cancer cells (micrometastases). Although it would be critical for such disseminated cancer cells to contain “cancer-initiating cells” (or cancer stem cells) for successful metastasis, whether they can expand or not will be highly dependent on the microenvironment (“the soil,” as Paget put it), and the wound-derived factors may affect the so-called metastatic “niche.”

Of importance, this inflammatory phase of wound healing is only temporary, lasting about 2 weeks postoperation. I propose that targeting this critical period of growth factor surge for therapeutic intervention is a promising approach for preventing metastasis, and, further, I plan to propose an actual protocol to do just this. In the subsequent remodeling phase, fibrosis and scar formation can take place in the wound. Of interest, similar reactions are also associated with tumor expansion, giving tumors a hard consistency that often makes them palpable. Once such reactions occur, tumors are physically protected from the surrounding tissues, and it would be difficult for therapeutic agents

Figure 1. Chronology of surgical wound healing. Note that time (horizontal axis) is in log scale. Modified from 27 and reprinted with permission.

Figure 2. Roles of bone marrow–derived cells (CD34+ Gr-1+ iMCs) in cancer invasion and metastasis.
and oxygen to reach them. This is one of the possible reasons that metastatic tumors are rather resistant to chemotherapy once they reach macroscopic sizes.

In the following discussion, I would like to use colon cancer as an example of a promising setting for metastasis prevention/control, although the basic concept for this approach should be applicable to other types of solid tumors, too. Most colorectal adenomas are initiated by inactivation of adenomatous polyposis coli protein (APC) in the colonic epithelium and progress to malignancy with additional genetic alterations involving Kras, p53, phosphoinositide 3-kinase (PI3K), and TGF-β receptor type 2 or SMAD4, as well as epigenetic changes in additional genes (14, 15). Genes whose mutations or epigenetic changes are responsible for invasion and metastasis of colon cancer, however, are only beginning to be unraveled.

**CCR1** - **Gr-1** immature myeloid cells and metastasis

A very promising approach for improving our understanding and, ultimately, control of invasion and metastasis involves the study of bone marrow–derived cells. Early work identified the activity of cancer cells in secreting CSF 1 and recruiting macrophages in breast cancer, activities shown to make the cancer more invasive and metastatic (8, 9). These findings had clinical implications that helped spur mechanistic studies of bone marrow–derived cell signaling, particularly in immature myeloid cells (iMC), and its inhibition.

As we reported in 1998 (16), knocking out Smad4 in the ApcΔ716 polyposis mouse model (17) leads to very invasive colorectal carcinoma. Our subsequent mechanistic studies of this aggressive phenotype (10, 18) showed that inhibition in the tumor cells of nuclear TGF-β signaling by Smad4 mutation causes increased expression of chemokine (C-C motif) ligand 9 (CCL9), which normally would be suppressed by TGF-β signaling. CCL9 binds to C-C chemokine receptor type 1 (CCR1) and thus recruits Gr-1+ iMCs, which produce matrix metalloproteinase 2 (MMP2) and -9 in contrast to the tumor cells, which do not, thus causing local invasion of the primary tumor. These results indicate that iMCs helped and promoted invasion of primary colon cancer.

More recently, we examined iMCs in the setting of liver metastasis (19). We injected colon cancer cells into spleens of syngeneic mice and let the cells disseminate to the liver. The cancer cells secreted CCL9, leading to a massive accumulation of iMCs, which was transient and disappeared in 3 weeks. Of importance, iMCs surrounded the tumor cells in the liver and helped in their colonization of liver tissue. Again, the micrometastatic tumor cells produced CCL9 (and produce CCL15 in humans), which bound to CCR1 on iMCs and recruited them to the sites of liver colonization. The CCR1 antagonist BL5923 blocked iMC accumulation and metastatic colonization and significantly prolonged survival of the host mice. Similar findings occurred with an antibody against CCL9 and in host mice with genetic knockout of CCR1, MMP9, or MMP2.

Two subsets of human colon cancer carry defects in TGF-β signaling. Most right-side colon cancers have mutations in TGF-β receptor type II (TGFBR2; ref. 20), whereas up to 35% of metastatic left-side colon cancers carry mutations in SMAD4 (21). Of interest, we found that half of the TGFBR2-mutant human colon cancers secrete CCL15 and recruit CCR1-positive iMCs. The fact that iMCs disappeared in full-blown large metastatic lesions supports this therapy in the adjuvant rather than metastatic setting.

Furthermore, the Moses lab (11) found that a different subset of iMCs (Gr-1+) plays similar roles in a breast cancer model with defective TGF-β signaling. They showed that a mutated TGF-β receptor recruits iMCs and the mechanism of recruitment involves C-X-C motif chemokine ligand 5 (CXCL5), whose receptor is CXCR2 receptor 2 (CXCR2), and involves MMP2 and -13. Therefore, invasion/metas- tasis models of our group (colon) and the Moses group (breast) illustrate that different iMC subsets promote invasion/metastasis via different chemokines and involve MMPs; both models also involve aberrant TGF-β signaling and MMPs.

Studies in murine models of lung tumorigenesis also found that high expression of CXCR2 ligands contributed to inflammation, neovascularization, and neoplasia, and that a CXCR2-neutralizing antibody was efficacious in reducing established tumors (22). A CXCR2-neutralizing antibody inhibited progression of premalignant alveolar lesions and induced apoptosis of vascular endothelial cells within alveolar lesions (23). The microenvironment was required for sensitivity to the antibody in both the malignant and premalignant preclinical studies. These results indicate that chemokines can regulate both early and late stages of lung tumorigenesis.

**Notch signaling and the invasion-metastasis cascade**

We recently identified the novel endogenous Notch-signaling inhibitor Aes as a metastasis suppressor (24). In another mouse model of colon cancer metastasis, where cancer cells were injected into the rectal smooth muscle layer, the cells metastasize to the liver, lungs, and lymph nodes at high frequencies. Expression of Aes was reduced significantly in the liver metastases compared with the primary tumors, which was also the case in human specimens.

Cell culture experiments showed a significant increase with Aes knockdown and decrease with Aes overexpression plasmids in the frequency of metastasis to the liver and lungs, whereas the frequency of metastasis to lymph nodes remained unaffected. Histologic immunofluorescence studies revealed a novel mechanism of Notch signal transcriptional repression by Aes: sequestration of Rbpj (CSL) by Notch. The Antitumor studies in primary human colorectal cancer xenografts showed suppression of colon cancer metastasis to the liver.

Studies in murine models of lung tumorigenesis also found that high expression of CXCR2 ligands contributed to inflammation, neovascularization, and neoplasia, and that a CXCR2-neutralizing antibody was efficacious in reducing established tumors (22). A CXCR2-neutralizing antibody inhibited progression of premalignant alveolar lesions and induced apoptosis of vascular endothelial cells within alveolar lesions (23). The microenvironment was required for sensitivity to the antibody in both the malignant and premalignant preclinical studies. These results indicate that chemokines can regulate both early and late stages of lung tumorigenesis.

**Notch signaling and the invasion-metastasis cascade**

We recently identified the novel endogenous Notch-signaling inhibitor Aes as a metastasis suppressor (24). In another mouse model of colon cancer metastasis, where cancer cells were injected into the rectal smooth muscle layer, the cells metastasize to the liver, lungs, and lymph nodes at high frequencies. Expression of Aes was reduced significantly in the liver metastases compared with the primary tumors, which was also the case in human specimens.

Cell culture experiments showed a significant increase with Aes knockdown and decrease with Aes overexpression plasmids in the frequency of metastasis to the liver and lungs, whereas the frequency of metastasis to lymph nodes remained unaffected. Histologic immunofluorescence studies revealed a novel mechanism of Notch signal transcriptional repression by Aes: sequestration of Rbpj (CSL) transcription factor to an insoluble nuclear matrix together with Aes, transducin-like enhancer 1 (TLE1; Drosophila Groucho homologue), and other cofactors as Notch intracellular domain (NICD), mastermind-like 1 (Maml1), histone deacetylase 3 (HDAC3), etc. Notch signal inhibition by 3 independent means all showed suppression of colon cancer metastasis to the liver.
and lungs: by Aes overexpression, by dominant-negative Rbpj at the transcription factor itself, and by a γ-secretase inhibitor (GSI) at upstream activation of the Notch receptor. These results indicate that inhibition of Notch signaling at any level can suppress colon cancer metastasis to the liver and lungs. Further studies with colon cancer cells placed on a human umbilical vein endothelial cell (HUVEC) layer in culture demonstrated that Notch signaling inhibition blocks transendothelial migration of the cancer cells, which is the cellular process involved in intravasation and extravasation of the metastasizing cancer cells. These data are also consistent with our finding that Notch ligands are expressed in tumor blood vessels and surrounding tissues. The latter include smooth-muscle, and liver and lung parenchymal cells (Fig. 3).

Moreover, a knockout mutation of the Aes gene in Apc intestinal polyposis mice showed dramatic local invasion and intravasation by the intestinal tumors. Taken together, these results demonstrate the significant role of Notch signaling in metastatic spreads of primary colon cancer cells and in metastatic target organs. The fact that Notch signal inhibition prevented early steps in the metastatic cascade (invasion, intravasation, and extravasation) supports this therapeutic strategy in the adjuvant setting rather than after metastasis is established.

It is worth noting that Notch signaling is involved in many processes of physiology and pathology, and some chemicals and antibodies have been developed to inhibit this signaling for therapeutic purposes. For example, GSIs have been tried in clinical trials to block amyloid plaque formation in Alzheimer’s disease, without success so far. Of interest, recently developed Notch receptor antibodies appear promising because unwanted adverse effects such as goblet cell metaplasia of the intestinal crypts can be avoided when Notch signaling inhibition is limited to a particular Notch paralog with specific antibodies rather than inhibiting all receptors together (25). Therefore, testable agents for this target are available for metastasis prevention trials.

Because Notch signaling inhibition can suppress early steps of the metastatic cascade including extravasation, it also fits in the metastasis prevention scheme that targets cancer cells disseminated to the liver and lungs in the postoperative inflammation phase of wound healing.

**Remaining challenges**

Although it would be ideal if therapeutic agents were available that block the growth and reduce the size of full-blown metastatic tumors, there are few such agents for solid tumors. A major difficulty for any potential such agents would be to penetrate into large macroscopic metastases that are tightly surrounded by fibrotic tissues. On the other hand, disseminated cancer cells at early stages of metastasis (such as those in the processes of invasion, intravasation, extravasation, and formation of micrometastasis) are likely more sensitive to therapeutic interventions. Indeed, it is already known that most of the numerous cancer cells shed into the circulation rarely form metastatic lesions, thus displaying metastatic inefficiency (26). Therefore, it is testable whether early processes of metastasis are more efficient in the inflammatory phase of postoperative wound healing and whether targeting these inflammatory steps of the “growth factor surge” can significantly reduce metastatic frequency.

As described by Virchow in 1863, cancer is a “wound that never heals.” Many types of inflammatory cells that are shared by surgical wound healing help to expand cancer tissues. Therefore, it is reasonable to therapeutically target the postoperative inflammatory phase to prevent metastasis. Therapeutic agents have better access to tumor tissues before the remodeling phase of wound healing and the attendant increase in fibrosis of metastatic tumors. Furthermore, there are some candidate agents that are available for testing.

From the standpoint of the pharmaceutical industry, however, this “metastasis prevention” framework may be an unfavorable design. To show statistically significant efficacy of a candidate agent, it would be necessary to
demonstrate a reduced recurrence rate by following up a large number of patients for a long period. Inevitably, this proof would be costly. For example, to show a significant reduction in the current relapse rate of approximately 50% following resection of liver metastases in colorectal cancer patients, it would require at least hundreds, possibly thousands of patients who would need to be followed for 5 years postoperation (in studies that could last up to 10 years). On the other hand, the pharmaceutical industry may view this framework and its logistics more favorably if it made use of compounds such as CCR1 inhibitors already developed for other diseases (such as multiple sclerosis or rheumatoid arthritis) and that appear to be clinically unsuccessful in the originally designed target diseases. The number of patients who die of metastasis should be justification enough to pursue the goal of metastasis prevention in the adjuvant setting.

Conclusions
As we stratify cancer patients into more subclasses based on their mechanisms of invasion and metastasis, we must be aware that at least some subsets are promoted by specific chemokines and/or signaling in the Notch pathway. These mechanisms are active in early stages of the metastatic cascade and are shared by surgical wound healing, especially in its inflammatory phase and overlapping proliferative phase (lasting about 4 weeks). To reduce the terrible consequences of metastasis, it is a worthwhile preventive approach to target this ‘growth factor surge’ with the most promising agents that have been developed previously for other diseases.

Disclosure of Potential Conflict of Interest
No potential conflicts of interest were disclosed.

Acknowledgments
The author would like to thank Kenji Kawada, Teruaki Fujishita, Takenori Kitamura, and Masahiro Sonoshita for discussions and encouragements. He also thanks Mikael Haggström for Fig. 1.

Received January 10, 2011; revised January 21, 2011; accepted January 24, 2011; published online March 3, 2011.

References
Cancer Prevention Research

Reflections on the Spread of Metastasis to Cancer Prevention

Makoto Mark Taketo


Updated version
Access the most recent version of this article at:
http://cancerpreventionresearch.aacrjournals.org/content/4/3/324

Cited articles
This article cites 24 articles, 8 of which you can access for free at:
http://cancerpreventionresearch.aacrjournals.org/content/4/3/324.full.html#ref-list-1

Citing articles
This article has been cited by 3 HighWire-hosted articles. Access the articles at:
/content/4/3/324.full.html#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.