



Published in final edited form as:

Cancer Res. 2016 June 01; 76(11): 3115–3117. doi:10.1158/0008-5472.CAN-16-1297.

Adhere, Degrade, and Move: the three-step model of invasion

Lance A. Liotta, MD, PhD

George Mason University, Virginia

Experimental work during the period 1975–1986 revealed the crucial importance of the extracellular matrix (ECM) in tumor cell invasion and metastasis, and culminated in the three-step hypothesis of invasion (1). The first step is tumor cell attachment to the ECM. The second step is proteolytic degradation of the ECM, led by advancing protruding actin rich pseudopods. The third step is migration of the tumor cell body through the remodeled matrix. This mechanistic scheme is widely accepted and continues to generate insights related to the large number of molecules, inside and outside invading cells, which all play a role in each of the three steps. Understanding the interaction of the tumor cells with the ECM has never been more clinically important. The ECM is not just a passive mechanical scaffold. Instead, the ECM is an active participant in neoplastic and physiologic invasion, and acts as an information highway, an immune sanctuary, and a storage depot supporting tumor growth and drug resistance.

Malignancy defined as the propensity to traverse basement membranes

When I started my career in cancer research in the late 1970s I was fortunate to choose the problem of invasion and metastasis. Today, forty years later, the clinical urgency of understanding metastasis has never been higher. For my PhD I asked: What are the rate limiting steps of metastasis? I studied the kinetics of circulating tumor cells and noted that only a tiny percentage of tumor cells entering the blood stream had the capacity to exit the blood vessel wall and initiate a metastatic focus. I hypothesized that crossing the continuous vascular sub endothelial basement membrane was a key rate-limiting step.(1,2) How did the tumor cell penetrate this physical barrier that does not appear to normally have pores large enough for cells to squeeze through? At the time, and still today, this question was not just relevant to tumor cell intravasation and extravasation. Basement membranes underlie epithelium, surround muscle, adipose cells and nerves, line body cavities, and comprise the dividing line between epithelial and mesenchymal compartments. Crossing of basement membranes is a rate limiting step for the transition from in situ to invasive carcinoma, and is required for invasion of all body tissue compartments. This general principle is now used by pathologists worldwide to diagnoses micro-invasion or to stage and gauge the aggressiveness of cancer.

Highly Influential Article cited for the AACR 75th Anniversary

Liotta, L.A. Tumor Invasion and Metastasis-Role of the Extracellular Matrix Rhoads Memorial Award Lecture. *Cancer Research* 46,1–7,1987

Proteolytic remodeling is required for traversal of basement membranes and ECM

A logical mechanism for tumor cell invasion of basement membranes was the secretion of degradative enzymes such as collagenase. In 1975, collagenase produced by the involuting tadpole tail (now called MMP1) was the only mammalian enzyme known to degrade stromal interstitial (Type I) collagen. It was suspected that the collagen found in basement membranes was a new and different type of non fibrillar collagen (now called type IV collagen). At that time the composition of basement membrane was being studied because of its role in diabetes and Goodpasture's syndrome. Basement membrane and extracellular matrix biochemistry had not yet been applied to cancer biology. I used a modified protocol provided by Nick Kefalides to isolate basement membrane collagen from mouse lungs. I found that metastatic tumor cells attached to, and degraded, the isolated basement membrane collagen (2). Working with George Martin and Shigeto Abe at the NIDDK in 1979 we found that classic tadpole MMP1 did not degrade basement membrane type IV collagen (3). The metalloproteinase that did degrade type IV collagen, which we purified from tumor cells (3), became known as MMP2. We found that higher levels of basement membrane, or extracellular matrix-degrading activity correlated with a higher number of metastasis in animal models (4).

Protease-mediated remodeling, or frank disruption of the basement membrane, is required for tumor cells, host cells, or embryonic cells that cross the basement membrane (1,5). The proteolytic enzymes that degrade the basement membrane during neoplastic invasion can be produced by tumor cells or contributed by host cells such as immune cells or endothelial cells. Vascular sprouts which utilize the same proteases and the same invasion associated molecules during angiogenesis (6). While MMP2 appears to be a crucial mediator of invasion (5,7) it is only one invasion-associated protease in a long list of MMP family members (7). MMPs are membrane bound or secreted, and can exist in a latent or active form bound to a variety of growth factors or inhibitors. MMPs functions extend beyond proteolysis to play a role in growth, signaling, and immune function. If MMP2, or other critical members of the MMP family are knocked down or inhibited, neoplastic, embryonic, or immune cell traversal of the basement membrane is prevented (5,8).

Three step model: Adhere, Degrade and Move

An explosion of new knowledge about the biochemistry of basement membranes and the generation of reagents for cancer research emanated from the discovery of the EHS sarcoma. This transplantable tumor was originally mislabeled as a chondrosarcoma because of the copious matrix it produced. Hinda Kleinman analyzed the EHS matrix expecting to find cartilage type II collagen. Instead, she found that the EHS tumor was actually producing all the basic components of basement membranes (9). The EHS sarcoma matrix, for the first time, provided biochemical quantities of basement membrane collagen, proteoglycans, and glycoproteins. This became the source material for the characterization of molecules crucial for the structure and function of all basement membranes including Laminin, Type IV collagen, and basement membrane proteoglycan. The unusual cross shaped structure of

Laminin (1), was characterized by rotary shadowing electron microscopy, and Type IV collagen was found to be a procollagen-like molecule that is essential for morphogenesis and organ shape. The layered, oriented set of molecules comprising the deformable and strong basement membrane, with its many biologic functions, is highly defined. Now called Matrigel, EHS tumor derived matrix has been critical to many fields of research studying the extracellular matrix. Matrigel is commonly used as an *in vitro* substrate for studying invasion (5).

The experimental data we gathered about tumor cell invasion of the extracellular matrix led to a unifying mechanistic 3-Step model of invasion (1, 10). The first step is adhesion of the invading cell to the basement membrane or other ECM (e.g. perivascular or sub epithelial stroma), Adhesion is mediated by one or more integrin family adhesion molecules. The second step is proteolytic degradation of the matrix by cell surface or locally active proteases that are localized at the tips of protruding actin-rich pseudopods (10). The pseudopods direct the proteolysis at the leading edge of the invading cell (1,5,10). The third step is de-adhesion and reversal of proteolysis by tissue inhibitor of metalloproteinases (TIMPs), and other mechanisms, so that the cell body is released to migrate forward into and through the pathway created by the pseudopod. Invasion is a complicated tightly regulated cyclical process of adhesion and de-adhesion, proteolysis and anti-proteolysis, and migration triggered by chemotaxis and haptotaxis. The source of the chemotactic molecules can be generated as a soluble gradient, or can be bound to ECM molecules in the solid phase. The successful invading cells must be able to coordinate all of these functions. Thus the machinery of invasion employed by cells is highly analogous to the Chunnel tunnel boring machine. A tunnel digger also use 3 steps to cut the rock at the front, while it cycles through the use of side braces, and then drives forward into the tunnel it has carved.

The adhere-degrade-move scheme is widely accepted (5,8). Today the structure of the invading pseudopodia has been largely elucidated, going far beyond our original simple observations in 1987 that proteases and chemotactic receptors were associated with actin rich protruding pseudopods (10). The mechanisms of actin nucleation and elongation have been further defined (5). Additional proteases, anti proteases, and adhesion molecules have been elucidated (7). Nevertheless, opinions differ on which integrins and which proteases are key for crossing basement membranes, interstitial matrices, or tissue structures such as bone and nerve, in a given case (5). In fact, different molecules within the 3-step paradigm may play a role depending on the context and tissue structure that is being invaded.

A view to the Future

ECM: immune sanctuary, and a highway for invasion

Invasion is the product of a close cooperation between the tumor and the host. The same 3 step mechanism is used during non neoplastic physiologic invasion. Invading tumor cells or tumor stem-like cells invade using mechanisms adopted from, or in cooperation with, normal cells. Examples of physiologic invasion include trophoblasts during placental implantation, neural cone directed neurite invasion during embryogenesis, breast epithelial cell degradation of the basement membrane during outgrowth and involution, and embryogenesis (7,8). Normal adult stem cells leave the bone marrow, extravasate, intravasate, and colonize

distant tissue; a phenotype that is identical to cancer metastasis. The difference between physiologic and malignant invasion is that physiologic invasion stops when the job is done. In contrast, tumor cells invade relentlessly, and are actively induced to invade during hypoxic and metabolic stress.

The ECM serves as a storage depot of growth factors, immune cell cues, and attractants or repulsing molecules. This creates a roadmap or a set of guideposts for the invading cells. The orientation, composition, and tensile properties of collagen fibrils, and basement membranes, direct cell migration in the embryo and in cancer. The ECM matrix is more than a barrier or a supporting scaffold (7) It is a dynamic structure that is intimately involved in tissue and organ development, cell and tissue orientation and size, immune cell function, healing, cell differentiation, and the storage of information. Basement membranes shield the emergence of malignant cells from recognition by the immune system. Immune cell effectors of tumor cell killing such as infiltrating T cells require direct physical cell-cell contact with the tumor cells. Pre invasive neoplastic lesions, arising for example in the breast, prostate, or pancreas, are surrounded by a continuous basement membrane. The basement membrane normally is a barrier to the passage of immunoglobulins and immune cells. Hidden behind the basement membrane cloak, the pre-invasive cells can persist and genetically evolve while they live in a sanctuary that shields outside immune cells from direct contact with the tumor cells.

Are invading cells drug resistant?

The process of invasion is dependent on the successful coordinated timing and activity of multiple tumor cell and host cell molecules. Any one of the necessary invasion molecules can be a target for a pharmaceutical compound that blocks invasion. Notwithstanding the fact that physiologic invasion is a critical part of normal host functions, what are the clinical indications for an invasion inhibitor? Despite hopes to the contrary, blocking the extravasation of circulating tumor cells is not a clinically useful goal. This is because, at the time of cancer diagnosis, the patient does, or does not, have distant metastasis. An invasion inhibitor may have no effect on the outgrowth of established metastasis, which don't require invasion for growth. Treating the patient at the time of diagnosis with an invasion inhibitor could block the tiny number of circulating tumor cells in the act of extravasation. Unfortunately this will not make much of a difference compared to the billions of previously circulating tumor cells discharged by the primary tumor prior to diagnosis.

Despite this pessimism, there is another exciting option for an invasion inhibitor that has not yet been tested. Invading tumor cells could be a source of drug resistant clones. An invading cell cannot migrate and divide at the same time (8). This means that tumor cells in the active state of invasion will be drug resistant at the time anti-proliferative therapy is administered. Hypoxia and metabolic stress generated by treatment stimulates invasion and will therefore increase the number of tumor cells that are not susceptible to further rounds of therapy. Using this argument, I can envision that cycles of an anti-invasion therapy administered prior to an anti-proliferative treatment will reduce the number of drug resistant invading clones that persist during treatment. In this way, anti-invasion therapies can potentially elevate the durability of anti-proliferative treatments.

References

1. Liotta LA. Tumor Invasion and Metastasis-Role of the Extracellular Matrix Rhoads Memorial Award Lecture. *Cancer Research*. 1987; 46:1–7.
2. Liotta LA, Kleinerman J, Catanzaro P, Rynbrandt D. Degradation of basement membrane by murine tumor cells. *J Natl Cancer Inst*. 1977; 58:1427–1431. [PubMed: 192901]
3. Liotta LA, Abe S, Robey PG, Martin CR. Preferential digestion of basement membrane collagen by an enzyme derived from a metastatic murine tumor. (MMP-2). *Proc Natl Acad Sci USA*. 1979; 76:2268–2272. [PubMed: 221920]
4. Liotta LA, Tryggvason K, Garbisa S, Hart I, Foltz C, Shafie S. Metastatic potential correlates with enzymatic degradation of basement membrane collagen. *Nature*. 1980; 284:67–68. [PubMed: 6243750]
5. Madsen C, Sahai E. Cancer Dissemination-Lessons from Leukocytes. *Developmental Cell*. 2010; 19:13–26. [PubMed: 20643347]
6. Kalebic T, Garbisa S, Glaser B, Liotta LA. Basement membrane collagen: Degradation by migrating endothelial cells. *Science*. 1983; 221:281–283.
7. Egeblad M, Werb Z. New functions for the matrix metalloproteinases in cancer progression. *Nature Reviews Cancer*. 2002; 2:161–174. [PubMed: 11990853]
8. Matus DQ, Lohmen LL, Kelly LC, ... Zang W, Chi Q, Sherwood DR. Invasive Cell Fate Requires G1-Cell Cycle Arrest and Histone Deacetylase-Mediated Changes in Gene Expression. *Developmental Cell*. 2015; 35:162–174. [PubMed: 26506306]
9. Kleinman HK, McGarvey ML, Liotta LA, Robey PG, Tryggvason K, Martin GR. Isolation and characterization of type IV procollagen, laminin, and heparin sulfate proteoglycan from the EHS sarcoma. *Biochemistry*. 1982; 21:6188–6192. [PubMed: 6217835]
10. Guirguis R, Margulies IMK, Taraboletti G, Schiffmann E, Liotta LA. Cytokine-induced pseudopodial protrusion is coupled to tumor cell migration. *Nature*. 1987; 329:261–263.