Tumor Cell Invasion—Not All Barriers Are Created Equal
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Abstract
The importance of invasion in the complex process of metastasis, although now well established, has been studied with increasing molecular detail due to the development of robust in vitro experimental assays. In this issue of Cancer Research, we highlight a paper published by George Poste and colleagues that compared and contrasted several different invasion assays. The authors concluded that various barriers impose different selective pressures and that simply screening for high-throughput screening of invasion (17).

In 1980, in contrast to many of the earlier studies, interpretation of many prior invasion studies was somewhat less certain in the absence of isogenic, related metastatic, and nonmetastatic pairs of cells. Fidler provided the entire metastasis research workforce such a pairing derived from the B16 melanoma (18), which was used in this article. Together, the paired cell lines allowed direct comparison of assays and complementary studies in vivo.

The first thing I noticed when rereading this article was the detailed Materials and Methods section. Unlike so many articles published today, the authors provided abundant details as well as details to watch for when someone would eventually attempt to replicate the experiments. The authors then went on to utilize these invasion barriers to repeatedly select for cells that invaded through them. As expected, repeated selection of highly invasive

Nowadays, with commercially available transmembrane invasion assays that can be used to measure the invasive capacity of cells, it is hard to believe that scientists in 1980 were still developing reproducible and robust experimental techniques to measure invasion. Highlighted in this issue of Cancer Research, an article by Poste and colleagues compared and contrasted a number of invasion barriers to see which barrier most faithfully recapitulated what would happen in metastatic cancer (1). As they clearly pointed out, each tissue barrier utilized presented different advantages and disadvantages.

To appreciate the impact of this article, one must look at what was known when it was published. Much of the hemogenous metastatic cascade, as we know it, has been inferred on the basis of anatomy and physiology. Abundant data highlight the importance of invasion into, and eventually out of, vascular compartments, but in 1980, the molecular details were still largely unknown. Only months before Poste and colleagues published this work, Martin and colleagues presented the first evidence of a tumor-derived collagenase (2) that eventually became defined as a matrix metalloproteinase (MMP). In subsequent years, an entire family of cancer-associated MMPs has been characterized (3–7). Yet, even knowing some of the molecular details, biologic assays to measure invasion and the importance of the various proteases were, and still are, required.

Mareel and colleagues developed an approach that involved the invasion of tumor cells into three-dimensional chicken embryonic heart pieces (8–10), and, although correlations were observed between invasion in their system and metastatic capacity, relevance of myocardium as a barrier was questioned as well as the labor intensiveness of histology, which in many ways precluded adaptation to high-throughput or semi-high-throughput screening. Although the chorioallantoic membrane had been used for a number of years (11, 12), those who utilized it as an invasive barrier recognized the variability of each membrane with regard to thickness, pliability, and other biophysical parameters. Other investigators adapted these methods to make them more amenable for high throughput screening in an attempt to discover antinvasive and hopefully antimetastatic therapies. For example, Gehlsen and colleagues and Hendrix and colleagues developed the membrane invasion culture system (MICS), which allowed investigators to compare side-by-side invasion across the same chorioallantoic membrane with different treatments (13, 14). This system was dramatically improved with the development of Matrigel, a reconstituted basement membrane isolated from the Engelbreth–Holm–Swarm sarcoma, by Kleinman and colleagues (15) and the utilization of Matrigel in this and a contemporaneous in vitro invasion assay by Albini and colleagues (16). Further modifications made the invasion assays more amenable for high-throughput screening of invasion (17).

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Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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subpopulations was possible through all of the barriers. Not unexpectedly, each barrier represented a different level of complexity and difficulty for the tumor cells.

Perhaps the reason this article has been so influential is the apparent discordance between highly enriched invasive cells and the ability to form metastases following orthotopic injection for intravenous injection, depending upon the selective pressure(s) imposed. Although not explicitly stated, I am relatively confident that the authors expected that all of the invasive variants would be more highly metastatic; that is not the result that they obtained, but they carefully and objectively revised their hypotheses in the context of experimental details regarding the methodology. Their report is refreshing in its candor and illustration of the experimental process.

The differences observed between invasiveness and metastatic competence may also provide clues regarding why subsequent clinical trials with MMP inhibitors did not fulfill their expected promise (4, 6, 19). In simplistic terms, we still do not fully understand the biologic barriers encountered by invading and circulating tumor cells.

The metastasis field has indeed come a long way since 1980, but the observations and methods developed in the early days established a foundation upon which further progress could be made. An important point illustrated in the article by Poste and colleagues is that the metastatic (and invasive) ability of cells combines properties from tumor cells as well as the barriers that they must traverse. The complex interactions between tumor cells and the different microenvironments in which they find themselves are not yet adequately recapitulated outside of an intact animal. It is worth highlighting that in vitro assays are suitable for exploration of some molecular details associated with cancer metastasis. However, caution must be applied to extrapolate in vitro results to metastasis as it occurs in vivo.

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

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