Published in final edited form as:

Cancer Res. 2016 April 1; 76(7): 1675–1676. doi:10.1158/0008-5472.CAN-16-0550.

Tumor cell invasion — not all barriers are created equal

Danny R. Welch

¹Department of Cancer Biology, The University of Kansas Medical Center, Kansas City, Kansas

²Department of Molecular & Integrative Physiology, The University of Kansas Medical Center, Kansas City, Kansas

³Department of Pathology & Laboratory Medicine, The University of Kansas Medical Center, Kansas City, Kansas

⁴The University of Kansas Cancer Center, The University of Kansas Medical Center, Kansas City, Kansas

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 enriching for invasive ability didn't necessarily translate into greater metastasis efficiency. Although perhaps obvious now, these findings were surprising when they were published. Certainly, the data highlight the importance of tumor cell-microenvironment interactions and the necessity to interpret experiments taking the context into consideration.

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*, a paper by George Poste, John Doll, Ian Hart and Josh Fidler compared and contrasted a number of invasion barriers to see which 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 paper, one must look at what was known when it was published. Much of the hematogenous metastatic cascade, as we know it, has been inferred based upon anatomy and physiology. Abundant data highlights 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, Lance Liotta and George Martin presented the first evidence for a tumor-derived collagenase (2) that eventually became defined as a matrix metalloproteinase. In subsequent years, an entire

family of cancer-associated MMPs have been characterized (3–7). Yet, even knowing some of the molecular details, biological assays to measure invasion and the importance of the various proteases were, and still are, required.

Marc Mareel and colleagues developed an approach that involved invasion of tumor cells into three-dimensional chicken embryonic heart pieces (8–10). And, while 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 CAM had been used for a number of years (11, 12), those who utilized it as 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 anti-invasive and hopefully anti-metastatic therapies. For example, Kurt Gehlsen and Mary Hendrix developed the Membrane Invasion Culture System (MICS), which allowed investigators to compare side-by-side invasion across the same CAM with different treatments (13, 14). This system was dramatically improved with the development of Matrigel, a reconstituted basement membrane isolated from the Englebreth-Holm-Swarm sarcoma, by Hynda Kleinman (15) and the utilization of Matrigel in this and a contemporaneous *in vitro* invasion assay by Adriana Albini (16). Further modifications made the invasion assays more amenable 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 non-metastatic pairs of cells. Fidler provided the entire metastasis research workforce such a pairing derived from the B16 melanoma (18), which was used in this paper. Together the paired cell lines allowed direct comparison of assays and complementary studies *in vivo*.

The first thing I noticed when rereading this paper was the detailed Materials and Methods section. Unlike so many papers 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 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 paper 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'm 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 didn't fulfill their expected promise (4, 6, 6, 19). In simplistic terms, we still don't fully understand the biological 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 paper 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*.

References

1. Poste G, Doll J, Hart IR, Fidler IJ. In vitro selection of murine B16 melanoma variants with enhanced tissue-invasive properties. *Cancer Res.* 1980; 40:1636–44. [PubMed: 7370995]
2. Liotta LA, Abe S, Robey PG, Martin GR. Preferential digestion of basement membrane collagen by an enzyme derived from a metastatic murine tumor. *Proc Natl Acad Sci U S A.* 1979; 76:2268–72. [PubMed: 221920]
3. Kessenbrock K, Plaks V, Werb Z. Matrix metalloproteinases: Regulators of the tumor microenvironment. *Cell.* 2010; 141:52–67. [PubMed: 20371345]
4. Fingleton B. Matrix metalloproteinases: roles in cancer and metastasis. *Front Biosci.* 2006; 11:479–91. [PubMed: 16146745]
5. Chambers AF, Matrisian LM. Changing views of the role of matrix metalloproteinases in metastasis. *J Natl Cancer Inst.* 1997; 89:1260–70. [PubMed: 9293916]
6. Vandenbroucke RE, Libert C. Is there new hope for therapeutic matrix metalloproteinase inhibition? *Nat Rev Drug Discov.* 2014; 13:904–27. [PubMed: 25376097]
7. Gross J, Lapiere CM. Collagenolytic activity in amphibian tissues: a tissue culture assay. *Proc Natl Acad Sci U S A.* 1962; 48:1014–22. [PubMed: 13902219]
8. Mareel MM, Van Roy FM, Messiaen LM, Boghaert ER, Bruyneel EA. Qualitative and quantitative analysis of tumour invasion *in vivo* and *in vitro*. *J Cell Sci.* 1987; 8:141–63.
9. Bracke ME, De Mets M, Van Cauwenberge RM, Vakaet L Jr, De Bruyne GK, Mareel MM. Confrontation of an invasive (MO4) and a noninvasive (MDCK) cell line with embryonic chick heart fragments in serum-free culture media. *In Vitro Cell Dev Biol.* 1986; 22:508–14. [PubMed: 3759793]
10. Mareel MM. Invasion *in vitro*. *Cancer Metastasis Rev.* 1983; 2:201–18. [PubMed: 6352015]
11. Hart IR, Fidler IJ. An *in vitro* quantitative assay for tumor cell invasion. *Cancer Res.* 1978; 38:3218–24. [PubMed: 688212]
12. Easty DM, Easty GC. Measurement of the ability of cells to infiltrate normal tissues *in vitro*. *Br J Cancer.* 1974; 29:36–49. [PubMed: 4820945]
13. Hendrix MJC, Seftor EA, Seftor REB, Fidler IJ. A simple quantitative assay for studying the invasive potential of high and low human metastatic variants. *Cancer Lett.* 1987; 38:137–47. [PubMed: 3690504]
14. Gehlsen KR, Wagner HN, Hendrix MJC. Membrane invasion culture system (MICS). *Med Instrum.* 1984; 18:268–71. [PubMed: 6493100]

15. Kleinman HK, McGarvey ML, Liotta LA, Robey PG, Tryggvason K, Martin GR. Isolation and characterization of type IV procollagen, laminin, and heparan sulfate proteoglycan from the EHS sarcoma. *Biochem.* 1982; 21:6188–93. [PubMed: 6217835]
16. Albini A, Iwamoto Y, Kleinman HK, Martin GR, Aaronson SA, Kozlowski JM, et al. A rapid *in vitro* assay for quantitating the invasive potential of tumor cells. *Cancer Res.* 1987; 47:3239–45. [PubMed: 2438036]
17. Welch DR, Lobl TJ, Seftor EA, Wack PJ, Aeed PA, Yohem KH, et al. Use of the membrane invasion culture system (MICS) as a screen for anti-invasive agents. *Int J Cancer.* 1989; 43:449–57. [PubMed: 2925275]
18. Fidler IJ. Selection of successive tumor lines for metastasis. *Nature New Biol.* 1973; 242:148–9. [PubMed: 4512654]
19. Coussens LM, Fingleton B, Matrisian LM. Cancer therapy - Matrix metalloproteinase inhibitors and cancer: Trials and tribulations. *Science.* 2002; 295:2387–92. [PubMed: 11923519]