Journal of Cellular Biochemistry 56:1–3 (1994)

VIEWPOINT

Orthotopic Is Orthodox: Why Are Orthotopic-Transplant Metastatic Models Different From All Other Models?

Robert M. Hoffman

AntiCancer, Inc., San Diego, California 92111 and Laboratory of Cancer Biology, University of California at San Diego School of Medicine, La Jolla, California 92093-0609

Metastasis of human cancer is a very complex process involving cell migration, invasion, seeding, adhesion, and all the processes involved in cell growth. In order to understand and treat metastasis, models that represent the human process are required.

Most current animal models employ athymic nude mice as hosts since these naturally avoid the obstacle of immunological rejection. However, until recently animal models have not yet fully achieved the promise of clinical representation.

The earliest nude mouse human tumor models were implanted with human tumor cells subcutaneously. These models first used established cell lines but later employed patient-derived material. Take rates from patient tumors varied considerably with tumor type and were always relatively low [Fidler, 1990]. The subcutaneous tumors generally depart markedly in their phenotype from the original. Most significantly important, these subcutaneous tumors very rarely metastasize in the mouse even when derived from a highly metastatic patient tumor [Fidler, 1990]. Thus, the subcutaneous tumors fail to reproduce an essential part of human tumor behavior. Even for the primary tumor, drug responses often did not match the clinical pattern.

Fidler [1990] noted that the subcutaneous micro-environment for human visceral tumors is radically different from their original milieu. He postulated that this difference may result in the lack of metastases and the altered drug responses seen in the subcutaneous models. Indeed radical differences have been noted by Fidler in the drug responses of tumors in the orthotopically transplanted site vs. metastases resulting from the orthotopically transplanted tumor vs. the tumor transplanted and growing subcutaneously [Wilmans et al., 1992]. Despite species difference, the corresponding nude mouse organ should more closely resemble the original micro-environment than the subcutaneous milieu. Injecting tumor cell suspensions into the analogous mouse sites gave striking results; for the first time the transplanted tumors resembled patient tumors and showed significant metastatic rates. For example, disaggregated human colon-cancer cell lines injected into the cecum of nude mice produce tumors that eventually metastasize to the liver.

Although orthotopic injection of cell suspensions is a marked improvement over simple subcutaneous implantation, the technique has several major drawbacks. Cell injection so far has been shown to work reliably essentially only with established cell lines which greatly restricts its utility [Fidler, 1990]. Also, the resulting tumors often showed relatively low rates of metastasis compared to the original. To obtain patient-like metastatic rates and patterns we invented surgical orthotopic implantation (SOI) of intact tissue [Fu et al., 1991a,b; 1992a,b,c, 1993; Fu and Hoffman, 1992, 1993; Wang et al., 1992a,b,c; Furukawa et al., 1993a,b,c,d,e,f; Hoffman, 1992; Kuo et al., 1992, 1993a,b; Astoul et al., 1993]. The results of SOI allowing patient-like metastatic rates and patterns appear quite general having been reproduced for bladder [Fu et al., 1991b, Fu and Hoffman, 1992], lung [Wang et al., 1992a,b; Kuo et al., 1993a; Astoul et al., 1993], stomach [Furukawa et al., 1993a,c,e], colon [Furukawa et al., 1993b,d; Fu et al., 1991a,

Received May 9, 1994; accepted May 10, 1994.

Address reprint requests to Robert M. Hoffman, AntiCancer, Inc., 7917 Ostrow Street, San Diego, CA 92111.

1992c; Kuo et al., 1993a], prostate [Fu et al., 1992b], pancreas [Fu et al., 1992a; Furukawa et al., 1993f], and ovarian cancers [Fu and Hoffman, 1993]. The mouse models constructed with SOI have been termed MetaMouse™.

The MetaMouse™ models described in this issue by Kubota and Astoul et al. demonstrate that the development of the new microsurgical procedures of (SOI) [Fu et al., 1991a,b, 1992a, b,c, 1993; Fu and Hoffman, 1992, 1993; Wang et al., 1992a,b,c; Furukawa et al., 1993a,b,c,d,e,f; Hoffman, 1992; Kuo et al., 1992, 1993a,b; Astoul et al., 1993] had a profound effect on the results achieved which allow for the first time tumors to replicate their clinical pattern in the SOI-transplanted nude and SCID mice. SOI seems to demonstrate that histologically-intact tumor tissue is important for the necessary intra- and inter-cellular reactions to occur that enable tumor cells to metastasize to their full capacity.

In this issue Dr. Price suggests that metastasis is not a random event, but rather the result of a sequence of selective events, many of which involve interactions with elements of the microenvironment of the primary and metastatic tumors. Analysis of the metastatic potential of a human tumor cell populations has been greatly improved by the introduction of orthotopic models of tumor growth and metastasis. The orthotopic-transplant models have demonstrated that implanting human tumor cells into the appropriate tissue in immunodeficient rodents can increase both tumor take and incidence of metastasis. The orthotopic models should be used to validate the identity of candidate metastasis-associated genes, and to determine the value of new forms of therapy.

Dr. Fodstad and Dr. Kjenniksen have stated in this issue that recent research has provided results that highlight the role of the microenvironment in determining important characteristics of the metastatic cells, including their degree of differentiation and sensitivity to drugs. Furthermore, evidence is presented that questions the general validity of the notion of clonal selection of metastatic cells, and whether the metastatic phenotype is acquired through the last of a series of mutational events occurring during tumor progression.

In this issue, Dr. Leighton states the biology of animal cells in culture is often studied in individual cells or in sheets of cells. The relevance of such studies to the intact animal is unclear, since the spatial conditions encountered by cells in animals is one of dense three dimensional masses of cells, with limits to migration, and with gradients both of diffusion of metabolites and of morphologic maturation. These spatial requisites have gradually been met in culture. Dr. Leighton's account describes sponge matrix culture for three-dimensional growth and unilaminar, bilaminar, and radial histophysiologic gradient cultures. Some of the common neoplastic abnormalities of surface epithelial tissues are considered.

In this issue, Dr. Kerbel suggests a linkage may exist between certain forms of acquired drug resistance and metastasis. Attention is also drawn to the fact that new linkages between metastasis and drug resistance may be uncovered by analyzing the ability of tumor subpopulations to acquire drug resistance after one or several previous exposures to chemotherapeutic drugs. Furthermore, ability to detect induced or acquired drug resistance in vitro may be strongly influenced by the types of assay used to detect and monitor drug resistance. In particular, three-dimensional cell culture systems may reveal acquired or induced "multicellular" drug resistance in situations where conventional two-dimensional culture systems do not. Use of three-dimensional culture systems may therefore reveal as yet undiscovered associations between the phenotypes of metastasis and drug resistance.

Dr. Denhardt and Dr. Chambers in this issue state that they have found Osteopontin (OPN) serves both in a cell attachment function and a cell signaling function via the $\alpha_{v\beta\xi}$ integrin. OPN can also reduce cell oxidant levels and inhibit the killing of tumor cells by activated macrophages and endothelial cells. They hypothesize that those cancer cells that produce OPN at elevated levels can suppress the oxidative burst, inhibit NO production, and thus protect themselves from killing by specific host cell types.

Dr. Lejeune et al in this issue, have found that recombinant tumor necrosis factor-alpha (rTNFα) has potent antitumor activity in experimental studies on human tumor xenografts. However, in humans, the administration of rTNFα is hampered by severe systemic side effects. The maximum tolerated dose in humans is at least 10-fold less than the effective dose in animals. Isolation profusion of the limbs (ILP) allows the delivery of high dose rTNFα, in a closed system with acceptable side effects. In a protocol with a regimen of rTNFα, chemo-

therapy, interferon and hypothermia, for melanoma-in-transit metastases (stage IIIA or AB), a 91% complete response compared with 52% after ILP with melphalan alone was observed.

Thus orthotopic-models and drug discovery strategies with the models is leading to potentially effective strategies for the therapy of metastasis. The ensuing years should see these new stratagies converted into consistent clinical efficacy.

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