Commentary

Carcinogenesis and Metastasis Now in the Third Dimension—What's in It for Pathologists?

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Conflicting Approaches to the Explanations of Carcinogenesis and Metastasis

Arguably, the last 5 decades have witnessed the primacy of the notion that carcinogenesis was due to the accumulation of genomic mutations affecting a single cell¹ that acquired the ability to proliferate indefinitely and generate a tumor. Throughout the years, those DNA mutations were thought to affect an increasing number of genes whose products (proteins) played regulatory roles in intracellular functions, causing one epithelial cell to become a tumor and eventually metastasize. Those gene mutations would affect the expression of multiple genes involved in the cell cycle,² apoptosis,³ and/or differentiation,⁴ genes whose identities have, so far, remained elusive. Researchers who in the 1930s and 1940s favored this somatic mutation theory (SMT) were further energized by the resolution of the DNA structure in the early 1950s and the promises that this event heralded. This gene-centric view favored the adoption of a narrow reductionist approach to the study of cancer thus localized to the cellular and subcellular level of biological organization.

Despite its popularity, the SMT has been challenged by epistemological considerations and by data.^{5–7} Often-times, these objections were dismissed by adopting new *ad hoc* explanations that temporarily rescued the credibility of this theory,⁸ not unlike the epicycles created by pre-Copernican scholars that made data fit the mistaken idea of a geocentric planetary system.⁹

It is seldom acknowledged, however, that early on researchers with a keen understanding of embryology, physiology, and pathology offered a sound alternative to the SMT. For instance, J. Needham¹⁰ and C.H. Waddington¹¹ in the 1930s, and later on J.W. Orr,¹² C.J. Dawe,¹³ D. Tarin,¹⁴ C.M. Nelson and M.J. Bissell¹⁵ and others looked at cancer as a problem akin to development gone awry, that is, a supracellular, tri-dimensional, emergent disease of tissue architecture. Finally, in the last decade an increasing number of groups

have generated data that both implicitly and explicitly favor switching the emphasis from searching for intracellular targets of carcinogens to instead looking at interactions among epithelial and stroma cells and the extracellular matrix as the determinants of normalcy and/or cancer.^{16–21} For the most part, these approaches have attempted to ontologically meld the SMT with the alternative notion that carcinogens target stromal cells (fibroblasts, adipocytes, mast cells, macrophages, and so forth) and the extracellular matrix (collagen, elastic and reticular fibers, ground substance, and so forth). Based on epistemological and pragmatic approaches, challenges have been aimed at the original SMT and to this recent synthetic approach.^{5,6,20,21}

In this issue of *The American Journal of Pathology*, while using a three-dimensional *in vitro* assay and a surrogate animal model, McDaniel and colleagues²² present evidence that favors the general notion that the stroma plays a major role in affecting the behavior of the mammary gland epithelium during normalcy, carcinogenesis, invasion, and metastasis. Their quest began after the observation that during postlactational involution of the mammary gland, the stroma displays many properties that are also observed during wound healing and inflammation. Because chronic inflammation is a crucial risk factor for development of gastric and colon cancers, they hypothesized that the remodeling stroma during postlactational involution of the mammary gland may also support the development of mammary cancer. Consequently, these researchers focused their interest on the hormonal and reproductive status of the host as determinants of the behavior of the stroma on the proliferative and invasive behavior of primary and metastatic tumor masses. First, they ob-

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served biochemical differences in the matrix composition of involuting and resting mammary glands. Next, they compared the *in vitro* behavior of human breast epithelial cells exposed to Matrigel (an extracellular matrix preparation derived from an ascitic rodent tumor) and to extracellular matrices from nulliparous young rats and from lactating mammary glands undergoing weaning-induced involution. Interestingly, McDaniel and colleagues²² found that nulliparous matrix promoted organization of normal ducts by the (probably) normal MCF12A human breast cells whereas Matrigel allowed only the development of hollow epithelial cell spheres (mammospheres). In contrast, matrix from involuting mammary glands did not favor the formation of ductal structures by MCF12A cells; instead, these cells organized into mammospheres similar to those observed using Matrigel as a substrate. In addition, the nulliparous matrix preparation tamed the invasive properties of MDA-MB-231 human breast malignant cells. In contrast, matrix from involuting mammary glands favored MDA-MB-231 invasiveness.

Perceptively, McDaniel and colleagues²² next evaluated the influence of the above-mentioned matrices on the metastatic properties of the MDA-MB-231 cells. For this purpose, these cells were mixed with matrix and injected orthotopically into the mammary glands of nude mice. In this way they tried to overcome the valid criticism that these are just *in vitro* quirks. The results showed an increased incidence of mammary gland metastasis in the liver, lung, and kidney when the MDA-MB-231 cells were mixed with the matrix from involuting mammary glands. Moreover, angiogenic responses and reactive stroma were most frequently observed in the tumor/mammary gland border of primary tumors that resulted from an inoculum of cancer cells mixed with the matrix extracted from the involution group. As the authors note, the fact that a small inoculum of cells mixed with matrix resulted in long-lasting effects on angiogenesis, reactive stroma formation, and the ability to metastasize suggests that the matrix possesses inductive effects that most likely operate epigenetically rather than through mutational events.

These data also shed light on epidemiological evidence showing that pregnancy, even at an earlier age, is associated with a transient increase in the risk of breast cancer.^{23–25} Indeed, the experiments reported in the article by McDaniel and colleagues²² suggest a potential for altered organization of the epithelium, enhanced invasiveness, and metastatic potential due to involution. Moreover, pregnancy-related cancer is associated with a poor prognosis due to higher incidence of metastatic disease, a phenomenon linked to the involuting matrix in the experiments by McDaniel and colleagues.²²

The approach adopted by McDaniel and colleagues²² is novel. It warrants being pursued under comparable and additional conditions using other surrogate models to extend and solidify the value of these important original data. Together with other data stemming from the use of these tissue-based approaches to carcinogenesis and metastasis, a fully clarified view of the multiple interac-

tions among the many components of the stroma and the epithelial cells in the mammary gland of rodents, and eventually in the human breast, should emerge.

Introducing Epigenesis in Carcinogenesis

While these additional data are being collected, some speculative options can be offered based on the data already at hand. An unbiased observer might conclude, for instance, that carcinogenesis and metastasis are prominently influenced by interactions stemming from extracellular protagonists that influence the proliferation pattern and the phenotypic properties of discrete tumor cells and these cells will form normal or carcinogenic tissue patterns under rules mostly dictated by their extracellular context rather than by their intracellular genetic designs. Altogether, these data do not readily fit into the SMT. More importantly, the evidence collected by McDaniel and colleagues,²² and that previously collected by others using rodent mammary gland, skin, and other surrogate models, indicate that an explanation of carcinogenesis and metastasis at the cellular or subcellular levels is incomplete, if not plain irrelevant.^{16,18–20,26}

Organisms build themselves, both in normalcy and disease, by using all of the resources available to them, including gene products and the intracellular, intercellular, extracellular, and external environments. This realization carries with it the implication, alluded to by Ernst Mayr²⁷ a quarter of a century ago that, for most biological phenomena, exploring levels of complexity lower than that at which the phenomenon of interest occurs, usually adds little to what was learned at the original level of inquiry. As an exemplifying analogy, understanding the structure of the muscle protein myosin has not significantly contributed to the explanation of how the heart works as a pump. Regarding cancer, the record shows that describing ever more elaborate gene mutation combinations, signal transduction anomalies and aberrant gene expression patterns is unlikely to add significantly to the explanation of a process that takes place at a level of organization that is higher than the one where these biochemical events occur.^{5,28}

Although the work of McDaniel and colleagues²² as well as others stresses the role of the supracellular level of organization, the cancer community, guided by its traditional reductionistic bent, is finding a new way to explain the neoplastic phenotype in the intracellular compartment by proposing that epigenetic changes, such as aberrant patterns of DNA and histone methylation and acetylation or chromatin organization, play a causal role in neoplasia.^{29–32} Research in these emerging fields of gene imprinting and regulation of gene activation by chromatin remodeling during development, carcinogenesis, and metastasis should be encouraged for their own sake. However, this option would again divert the attention of cancer researchers toward a reductionist resolution of a disease whose explanation appears, instead, to be at the tissue level of biological complexity. In this regard, it is worth remembering the sober words of John Cairns³³ who wrote some years ago, "Biology and cancer research have developed together. Invariably, at each

stage, the characteristics of the cancer cells have been ascribed to some defect in whatever branch of biology happens at the time to be fashionable and exciting." Thus, it is not surprising that experimental data that cannot be explained by DNA mutations in the context of the SMT might be interpreted as suggesting the involvement of epigenetic mechanisms related to gene transcription. This new epicycle still points to the interior of an epithelial cell that may eventually become a tumor.

Future Prospects

Are the current and the above-referred compelling contributions a part of an incoming trend in which the SMT is being slowly and silently abandoned as a productive approach to explain cancer in favor of the soft landing of a research program lodged at a higher hierarchical level of organization, namely, that of tissue-tissue interactions? Will cancer-funding agencies recognize this trend and support it with an enthusiasm comparable to that afforded to the now tattered SMT? In any case, will this new approach improve the chances for patients to be offered more efficient treatments than the ones now available? Experienced observers in the field of science at large, and cancer research in particular, usually recommend being cautious before dismissing old paradigms or accepting new ones. However, now that it is being more readily accepted that the genome is not the driver of development³⁴ and that organicist views are again being appreciated in biology, it would probably be timely to extend these notions to the study of carcinogenesis and metastasis into organs other than the mammary gland.

Finally, contributions by any member of the scientific community to unravel the cancer puzzle should be welcomed by everyone. Nevertheless, the case for pathologists to assertively participate in this field is now compelling. Sufficient experimental and clinical data have been accumulating during the last few decades to warrant their much more active participation in this field of research. It is high time to acknowledge the merits of the organicist views on cancer elegantly articulated by D.W. Smithers³⁵ in the 1960s and mostly ignored by the reductionists. In short, the contributions of pathologists, ie, trained organicists, to cancer research at large and carcinogenesis in particular should be encouraged and welcomed. The current contribution by McDaniel and colleagues in this issue of *The American Journal of Pathology*,²² together with other recent findings referred to above, speak favorably in this regard.

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