Is Breast Tumor Progression Really Linear?

Commentary on Allred et al., p. 370

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“Progress has not followed a straight ascending line, but a spiral with rhythms of progress and retrogression, of evolution and dissolution.”

Johann Wolfgang Von Goethe

In real life, most of us are likely to agree with this quote from Goethe. Yet, our generally accepted view of tumor progression depicts a linear route going from a normal cell to a metastatic tumor driven by progressively accumulating genetic, epigenetic, and microenvironmental alterations. Tumorigenesis has been described as an evolutionary process decades ago (1,2), but still very few molecular studies have even attempted to analyze tumor progression from a population biology point of view. This is in part due to our relentless desire to view the world through a “simplifier glass” and in part due to the technical difficulties associated with these types of studies. Analyzing tumors not just as a ground-up bulk tissue, but as a population of individual tumor cells, requires the dissection of molecular differences at the single cell level or at least in homogenous cell populations. In this issue of Clinical Cancer Research, Allred et al. (3) have taken on the challenging task of evaluating intratumoral and intratumoral diversity in ductal carcinoma in situ (DCIS) of the breast using various approaches.

The currently accepted view of breast tumor progression assumes the gradual step-by-step transition of ductal hyperplasia to in situ then invasive and eventually metastatic carcinomas (Fig. 1; refs. 4–6). Thus, DCIS is considered the obligate precursor of invasive ductal carcinomas (7). This tumor progression model is strongly supported by epidemiologic, pathologic/clinical, and molecular data obtained in human breast cancer patients as well as in animal models. Premalignant tumors, including DCIS, are more frequently observed in women with a high risk of breast cancer and they are frequently located adjacent to invasive carcinomas (8,9). In addition, molecular studies have shown clonal relationships between tumors of different stages including DCIS and adjacent invasive cancer and DCIS and its invasive recurrence (10,11).

Breast cancer has long been recognized as a heterogeneous disease with varying clinical outcome. This intratumoral heterogeneity was dramatically shown by recent molecular profiling studies clustering the tumors into distinct luminal, HER2, and basal-like subtypes based on their global gene expression patterns (12,13). Luminal tumors are more differentiated, hormone receptor–positive, and in general, have better outcome. HER2+ tumors have amplification of the ERBB2 oncogene and respond to therapy targeting this receptor kinase. Basal-like tumors are poorly differentiated, lack hormone receptors and HER2, and in general, tend to have worse clinical outcome because of their propensity to develop distant metastases and lack of targeted therapy against them. Just as this new molecular classification system was established, however, it also became apparent that tumors within each subtype are still fairly heterogeneous with respect to clinical outcome and that some tumors do not fit into any of these major molecular subgroups. Thus, even this molecular-based classification is oversimplifying reality and further refinements are necessary.

To address intratumoral heterogeneity in DCIS at the molecular level, Allred and colleagues (3) analyzed histologic differentiation and commonly used prognostic biomarkers including hormone receptors (estrogen receptor and progesterone receptor), HER2, p53, and cell proliferation (Ki67 expression) in pure DCIS, DCIS adjacent to invasive cancer, and in invasive ductal carcinomas (200 cases of each tumor type). A subset of DCIS cases were also analyzed for global gene expression profiles. Overall, the distribution of histologic differentiation grades and their association with prognostic markers were the same in DCIS, DCIS adjacent to invasive ductal carcinoma, and in invasive ductal carcinoma. Correlating with previous data, well-differentiated tumors were more frequently hormone receptor–positive (estrogen receptor–positive and progesterone receptor–positive) and negative for HER2, p53, and Ki67 compared with poorly differentiated tumors. Unsupervised clustering of 25 DCIS tumors also identified the same luminal, basal, and HER2+ subtypes as has been previously reported in both invasive and in situ breast carcinomas (12,14).

When the researchers further refined their analysis to address intratumoral heterogeneity at the cellular level, however, they found that about half of the tumors were phenotypically highly diverse. This was true for histologic differentiation grades (e.g., the same DCIS show poorly and well-differentiated areas) as well as for the expression of several biomarkers analyzed (ER, HER2, GATA3, CK5/6, CK18, and p53). Furthermore, the expression of p53 (reflecting mutant p53) was statistically significantly correlated with the presence and extent of this phenotypic diversity. Molecular classification studies have suggested that distinct breast cancer subtypes might have distinct cells of origin and tumor progression pathways. The data presented by Allred and colleagues (3) indicates that this

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may not be so simple and generally true, however, as multiple tumor subtypes apparently frequently coexist within the same tumor. What is the reason for this intratumoral heterogeneity and what are its potential clinical implications?

Two of the currently prevailing models explaining intratumoral heterogeneity are the cancer stem cell and the clonal evolution hypotheses. According to the cancer stem cell hypothesis, a subset of cancer cells have the characteristics of stem cells and could give rise to progeny with different differentiation states (15, 16). The clonal evolution model, on the other hand, explains heterogeneity as a consequence of genomic instability, resulting in the continuous acquisition of new somatic changes, combined with the clonal selection for tumor cells with the most beneficial phenotype (1, 2, 17). In principle, both of these models are in agreement with the observations reported by Allred et al. (1). A limitation of the cancer stem cell hypothesis, however, is that it restricts the tumor progression driving events to the cancer stem cells, which does not seem to be advantageous from a tumor evolutionary point of view and cannot explain certain clinical data, such as the emergence of drug-resistant clones after treatment. In evolutionary algorithms, the fitness of a population increases with time by mutating and recombining individuals and by a biased selection of more fit individuals. The right selection pressure is critical in ensuring sufficient optimization progress and in preserving genetic diversity to be able to escape from local optima. How can we translate this knowledge in evolutionary biology and population genetics into tumor biology? How can we measure diversity within human tumors, and most importantly, what is the clinical relevance of intratumoral diversity?

The most comprehensive study addressing these issues in human tumors was conducted by Maley and colleagues focusing on a premalignant lesion known as Barrett’s esophagus (18).

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**Fig. 1.** Schematic outline of breast tumor progression. Tumorigenesis goes through defined histologic and clinical stages and is driven by progressively accumulating genetic, epigenetic, and microenvironmental alterations. Thin and thick black circles indicate the breast and the basement membrane surrounding the ducts, respectively. Myoepithelial/basal cells (green cells) synthesize and are in direct contact with the basement membrane, whereas luminal epithelial cells (red cells) lay on top of the myoepithelium. A major distinguishing feature of the in situ to invasive carcinoma transition is the disappearance of the basement membrane and the myoepithelial cell layer.

**Fig. 2.** Models of breast tumor progression. The linear evolution model proposes that there is a dominant tumor cell clone that progressively accumulates genetic and epigenetic alterations and it has growth and survival advantage due to environmental selection pressure. The diversity evolution model, on the other hand, proposes that although there could be a predominant clone within tumors, there are multiple other clones present as well. Depending on the selection conditions provided by the environment or therapeutic interventions, the identity of the dominant clone may change as tumors progress. As a consequence, heterogeneous tumors with higher clonal diversity are more likely to progress and develop resistance to treatment. Different color circles indicate distinct clones, whereas darkening intensity of the color reflects changes in the same clone (e.g., pink — red — dark red circles).
They dissected these premalignant lesions into 1-cm pieces and analyzed each of them independently for DNA content and genetic changes, including mutations in TP53 and CDKN2A and loss of heterozygosity at multiple loci. Based on these comprehensive molecular profiles, and the principles of population biology, the researchers defined a clonal diversity score for each tumor and analyzed the associations of these scores with clinical outcome. The overall conclusion of the study was that higher clonal diversity predicts the risk of progression to invasive cancer. Thus, similar to what has been observed in ecological populations, diversity is beneficial for tumor progression as well. Furthermore, correlating with the findings of Allred et al. (1), diversity was statistically significantly associated with the presence of mutant p53.

Although Allred et al. (1) has not analyzed the genetic clonality within DCIS, immunohistochemical staining for HER2 and p53 is likely to reflect gene amplification and mutation, respectively. Thus, DCIS tumors may also be genetically diverse as has been observed in Barrett’s esophagus and this diversity may also correlate with the risk of progression to invasive cancer. Thus, instead of the simple linear view of breast tumor progression, we may have to consider a revised view that incorporates clonal diversity as one of the driving forces of progression (Fig. 2). Recent in situ analysis of genomic instability during breast tumor progression using fluorescence in situ hybridization has shown a dramatic increase in chromosomal aberrations in DCIS compared with ductal hyperplasia possibly caused by telomere shortening–induced crisis (19). Combined genotype-phenotype studies can be conducted in DCIS by using immuno–fluorescence in situ hybridization and genetic mutations can be analyzed by using DNA from cells purified with immuno–laser capture microdissection (20). Performing these studies in DCIS with long-term clinical outcome will allow the determination if the risk of progression correlates with a specific genetic alteration or with clonal diversity. Because all the tools are available, this author is sure that we do not have to wait long before we know the answers to these questions.

References
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