Molecular Biomarkers of Risk in Premalignancy and Breast Cancer Prevention

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Abstract

About 50,000 U.S. women are diagnosed with breast atypical hyperplasia each year, giving them about a six-fold increased relative risk of developing invasive breast cancer (IBC) compared with age-matched controls. Still, only a small fraction of patients with atypical hyperplasia ever progress to IBC, which is a major reason why a large majority do not participate in breast cancer prevention, despite the remarkable effectiveness of currently available risk-reducing therapies. An interesting study reported by Radisky and colleagues in this issue of the journal (beginning on page 1953) evaluated expression levels of p16\textsuperscript{ink4a} in atypical hyperplasia for more accurately predicting risk—hoping to identify high-risk patients who will benefit most from therapy while sparing those with lower risk from unnecessary therapy. Unfortunately, p16\textsuperscript{ink4a} expression was not prognostic in this particular study, although research to identify powerful biomarkers of risk remains a high priority. Fortunately, there are many other promising biomarkers under investigation, as well as several underutilized experimental strategies which could help promote successful breast cancer prevention. Cancer Prev Res; 4(12); 1947–52. ©2011 AACR.

About 50,000 women are diagnosed with atypical hyperplasia each year in the United States (1, 2). It is usually an incidental finding in a core or excisional biopsy conducted for other reasons (e.g., mammographic calcifications). Women with atypical hyperplasia have about a 6-fold increased relative risk of developing invasive breast cancer (IBC) compared with age-matched controls, so the stakes are high if they are not diagnosed and appropriately treated (2, 3).

An interesting study reported by Radisky and colleagues in this issue of the journal looked at the relationship between p16\textsuperscript{ink4a} expression and the risk of developing IBC in patients diagnosed with atypical hyperplasia (4). These investigators carefully evaluated p16\textsuperscript{ink4a} expression by immunohistochemistry on excisional biopsies containing atypical hyperplasia from 233 women with long-term follow-up (median = 14.5 years). They previously found significantly increased risk associated with elevated COX-2 and Ki67 in atypical hyperplasia in the same cohort (5, 6). In the current study, however, they found no association between p16\textsuperscript{ink4a} and risk, whether considered alone, or in combination with COX-2 and Ki67.

In another recent study, Kerlikowske and colleagues observed that the elevation of all three of these biomarkers in patients with ductal carcinoma in situ (DCIS) treated by lumpectomy alone was associated with a significantly elevated risk of local recurrence of IBC (7, 8). On face value, the Radisky study seems at odds with this finding because atypical hyperplasia is considered to be the main precursor of CIS. However, there are two major subtypes of atypical hyperplasia, referred to as atypical ductal hyperplasia (ADH) and atypical lobular hyperplasia (ALH), and their incidences are about equal. There are also two major subtypes of CIS, referred to as DCIS and lobular CIS (LCIS), and only ADH is considered to be the primary precursor of DCIS. Radisky did not provide separate analyses of p16\textsuperscript{ink4a} in ADH and ALH, which may be important. In addition, the endpoints of the two studies were very different, making their direct comparison unwise, especially because recurrences following lumpectomy for DCIS are so heavily influenced by nonbiologic factors such as the status of surgical margins. Therefore, we have learned that p16\textsuperscript{ink4a} is not a biomarker of risk in the Radisky and colleagues group of patients with atypical hyperplasia, but as the authors cautioned, additional studies are required to settle this important issue.

The investigators involved in the aforementioned studies are leaders among a relatively small group who are working hard to promote research in molecular prevention, including searching for powerful new biomarkers of risk. This Perspective will briefly consider a few general issues which might help promote molecular research in the prevention of human breast cancer, especially in the short term.

First, progress in any type of prevention research will require substantially more support. There is currently an enormous imbalance in translational breast cancer research favoring therapy over prevention. For example, a National Cancer Institute (NCI) website for helping patients and
their doctors in finding open clinical trials recently listed 434 breast cancer treatment but only 26 breast cancer prevention trials (9). The logical outcome of this imbalance is that we may eventually be able to cure our daughters' breast cancers, but we will never be able to prevent them from getting the disease in the first place, which is ludicrous.

Research in prevention is relatively unpopular for many reasons, some true and significant, others not. Commonly cited reasons include that it is too difficult, slow, inefficient, expensive, challenging to translate into clinical trials and practice, and scientifically unexciting. Opinions and biases aside, epidemiologic studies involving migrating populations, diet, and exercise, to name a few, have unequivocally showed that breast cancer is fundamentally a cultural and preventable disease and that existing prevention strategies can be remarkably effective in relatively short periods of time (10–12). It took just 6 years to show the extraordinary effectiveness of tamoxifen in reducing breast cancer in high-risk women, which rivals the success of any therapeutic trial of adjuvant tamoxifen in women with breast cancer (13, 14).

No one should be advocating for less research in therapy, but the enormous genetic damage in breast cancers revealed by recent whole genome sequencing studies is a chilling indication of just how difficult it will be to develop effective targeted therapies (15, 16), far more difficult than originally anticipated. Everyone should be advocating for more research in breast cancer prevention, and many of us share the opinion that prevention is more achievable than cure.

Research to discover powerful biomarkers of breast cancer risk is one of the most important and promising areas for significant progress in breast cancer prevention in the near future. Current strategies to reduce risk are often perceived by women and their doctors as being too risky and onerous, despite their effectiveness, and only about 25% (or less) of higher risk candidates participate (17, 18). Biomarkers which improve on current methods of predicting individual risk might improve the uptake of prevention therapy by increasing the motivation of women at high risk to lose weight, improve their diet, exercise, take U.S. Food and Drug Administration–approved agents (e.g., tamoxifen and raloxifene), and so on. Understanding more about psychosocial predictors of patient adherence to effective prevention strategies could also help improve uptake (19). None of this is news, and several promising biomarkers are under active investigation (11, 20), although most are being evaluated in women already determined to be at a high risk and requiring treatment, such as those diagnosed with ADH and DCIS. Most breast cancers will develop in women with apparently "normal" risk, and finding biomarkers applicable in this setting should be a priority.

Searching for new biomarkers of risk in samples of apparently normal and premalignant breast tissue is a reasonable strategy at present because these samples are so commonly obtained from women at a potentially high risk for reasons leading to a biopsy (e.g., mammographic abnormalities). Although the premalignant lesions themselves are risk factors, only a small fraction ever progress to cancer (especially early lesions like hyperplasias) and the biomarkers being searched for are presumably linked to the underlying molecular reasons for tumor progression. This approach, however, has been a significant barrier to progress in the sense that most such investigations so far have relied on tissue samples removed in the distant past from noncancerous breasts of women who were retrospectively followed to determine the incidence of breast cancer. The major flaw of this design is that many (perhaps most) of the lesions under study were removed by the initial biopsy or follow-up surgery or were cured by adjuvant therapy (e.g., radiation for DCIS following lumpectomy), and so the connection between their biomarkers and subsequent cancer is tenuous. In addition, the incidence of cancer associated with most premalignant lesions is so low that the large numbers of samples required for adequate statistical power to support biomarker studies of risk are very difficult to obtain, which is why there is only a handful of sizable tissue resources like this (including those used in the Radisky and colleagues and Kerlikowske and colleagues studies) in the world—and this scarcity is unlikely to improve.

The scientifically most efficient study design would be to obtain samples of premalignant lesions from small biopsies (e.g., needle cores), knowingly leaving a portion of the lesions behind in the breast and then follow the patients without further therapy. Obviously, this would be unethical, although almost everything we know about the natural history of DCIS is based on studies that retrospectively identified DCIS in biopsies which were mistakenly diagnosed initially as benign and then ascertained the incidence of breast cancer in these patients (21, 22). Even though most of the mistakes involved small, low-grade DCIS, more than 30% of the patients eventually developed IBC in the region where the biopsy came from, representing a 10- to 12-fold increased relative risk versus age-matched controls. The true rate of IBC in women with undetected DCIS is probably higher because nearly all the samples involved in these studies were excisional biopsies large enough to completely remove many of the lesions.

Another useful and ethical design involves evaluating premalignant lesions which were not detected until after the patient developed IBC and which are most likely the lesions commonly found in cancerous breasts (23, 24); obtaining samples of this nature is relatively easy. Some of these premalignant lesions are probably the ancestors of the adjacent IBCs and, thus, had a 100% chance of progressing. Comparing them with histologically similar premalignant lesions from noncancerous breasts is essentially a case-control study design with strong ability to identify biomarkers of risk. It is arguably the best design currently available, but unfortunately it is rarely used. It also is applicable to the full range of premalignant breast lesions, and biomarker research could benefit by using it more often.

Almost all molecular studies in breast cancer prevention so far, ranging from biomarkers of risk to targeted therapy, have been based on previous observations from research in breast cancer, which seems backward. For example, the idea of using tamoxifen for prevention in high-risk women came...
from the reduction of contralateral risk observed in patients with breast cancer treated with adjuvant tamoxifen (in addition to some preclinical prevention data). In the future, however, successes in fundamental molecular prevention will most likely come from studies focusing on the forward development and progression of premalignant breast lesions to cancer.

Understanding more about the molecular physiology and biology of the normal breast will be extremely important because understanding departures from the norm will help in defining carcinogenic changes. Normal and abnormal features in human breast tissue can be extraordinarily complex, which is largely unappreciated. For example, Fig. 1 shows expression patterns of several genes at the protein level evaluated by immunohistochemistry in cells within a normal terminal duct lobular unit and an adjacent columnar cell hyperplasia (CCH), which is an important early premalignant lesion (25). The specific proteins include estrogen receptor (ER), cytokeratin 18 (CK18), CK5/6, cluster of differentiation 24 (CD24), CD44, vimentin, and aldehyde dehydrogenase (ALDH1), which are interesting because they are thought to play important roles in cellular differentiation and stem cell biology. Each protein shows complex patterns of expression in normal cells, which must be highly regulated. In contrast, some are very different in the adjacent CCH, such as elevated ER and vimentin in luminal cells. Identifying and understanding the causes of these early changes may lead to highly effective prevention strategies and should also be a research priority.

CCHs are extremely common and relatively uniform histologically and are considered by many experts to be the earliest histologically identifiable premalignant lesion in the breast (25). The increased ER and growth (i.e., hyperplasia) observed in CCHs compared with terminal duct lobular units suggest that exposure to elevated levels of estrogen may be important in the development of CCH (26). Consistent with this suggestion, female macaque monkeys rapidly develop CCH-like lesions after prolonged exposure to physiologic levels of estrogen, which is preventable with tamoxifen and reversible when the hormone is withdrawn (27, 28). The reason for elevated ER in CCH is unknown, but it may be fundamental to its development. It

Figure 1. Diversity of differentiation and stem cell biomarkers in normal and hyperplastic breast tissue. H&E, hematoxylin and eosin; TDLU, terminal duct lobular unit; VIM, vimentin.
may be possible to inhibit or reverse the development of CCH on a large scale by reducing the effects of estrogen exposure through diet, exercise, pharmacologic agents such as tamoxifen, or maybe even selectively manipulating the intestinal microbiome to suppress estrogen metabolism. CCHs are essentially the "fertile soil" for growing more advanced premalignant breast lesions and thus are another logical priority for molecular prevention research.

A new and potentially important early premalignant lesion, referred to as crown-like structures (CLS), has recently been identified in women and modeled in mice (29). CLSs are associated with obesity, inflammation, and locally increased aromatase expression and activity (29), all of which may be involved in their development. The aromatase associations have important clinical implications because they can be targeted for prevention with aromatase inhibitors.

Progression beyond hyperplasia is most likely driven by the acquisition of permissive mutations, which may explain why only a very small fraction of CCH appears to progress to atypical hyperplasia and beyond. The technology is becoming available to sequence the entire genomes of premalignant breast lesions, which may help reveal key driver mutations. These mutations should be relatively rare and, thus, theoretically easier to target therapeutically than the huge number of mutations found in all IBCs. The primary phenotypic consequences of mutations in atypical hyperplasia and CIS include evolution from a polyclonal (CCH) to monoclonal population of tumor epithelial cells, which detach from the basement membrane and grow on top of each other, distending ducts and lobules. Interestingly, the growth rates of CCH, atypical hyperplasia, and low-grade CIS are similar and quite low.

Comparative studies of the transcriptomes of CCH, atypical hyperplasia, and CIS may also be helpful in identifying key mutations and detrimental downstream consequences. Although the technology required for this work has been available for years, studies of this nature are rare to nonexistent. The primary barrier is that most of the material available to study is in the form of very small formalin-fixed paraffin-embedded tissue (FFPET) samples. Expression profiling of FFPET samples is difficult but possible, however, and it has been conducted at least once in samples of CCH compared with normal cells (30). Samples of atypical hyperplasia are particularly small and rare, but this should not be a major impediment to research because low-grade CIS is an excellent surrogate for atypical hyperplasia, and samples of CIS are relatively large and common. Distinguishing passenger from driver mutations will require functional studies involving relevant human premalignant cell lines, which are currently extremely scarce.

If becoming a breast cancer results from randomly acquiring multiple mutations enabling malignant behaviors (growth, motility, invasion, etc.), why is there an apparent order to the development and progression of premalignant lesions? For example, the incidence of premalignant lesions in randomly selected noncancerous breasts drastically declines from CCH to atypical hyperplasia to CIS, and one can find regions of gradual histologic continuity between them (24). Furthermore, premalignant lesions in cancerous breasts are much more common than in noncancerous breasts, the frequency of shared mutations with adjacent IBC increases in the direction of CCH to atypical hyperplasia to CIS (31, 32), and essentially all IBCs are admixed with DCIS (31). One of the main enigmas of breast cancer evolution is that random mutations appear to result in orderly tumor progression. Nonetheless, studying premalignant lesions as they are currently defined remains our best option for understanding the evolution and prevention of breast cancer because there are almost no data supporting alternative hypotheses—but it is probably a good idea to keep an open mind.

If contrasting normal cells, CCH, and atypical hyperplasia (or low-grade CIS as a surrogate of atypical hyperplasia) to IBC is a good strategy to reveal driver mutations, then contrasting DCIS to IBC is a good strategy to identify specific drivers of tumor invasion. The latter is by far the most advanced area of molecular research in breast cancer evolution. About 10 studies have directly compared the transcriptomes of human DCIS with IBC, each identifying at least a few differences, which are potentially important in regulating tumor invasion (examples in refs. 33–35). A concerted effort to identify overlap between these studies (e.g., a meta-analysis) might be helpful in pointing to particularly important alterations, although additional comparative studies are still desperately needed. Progress in sequencing the genomes of IBCs has identified a large number of mutations which could be resequenced in premalignant lesions in the same breasts to help identify driver mutations. In addition, there are at least a couple of human DCIS-like cell lines widely available to the research community (DCIS.COM and SUM225) to help confirm the functional significance of specific mutations, although this is another area for improvement. A recently developed xenograft model of DCIS based on injecting human DCIS cells directly into the nipple duct of immunodeficient mice could greatly improve our ability to confirm the functional significance of potentially important genes and mutations (36).

Genetic instability at any stage in the evolution of premalignant lesions to breast cancer would accelerate the accumulation of important mutations. Recent studies reveal that at least half of all DCIS contain substantial internal molecular diversity (31), which is often associated with defects in DNA repair genes (31), and the degree of diversity is directly related to tumor progression and clinical aggressiveness (37). There are probably subtle inherited differences between normal individuals in their ability to repair damaged DNA, which may influence their lifetime risk of developing breast cancer. McDermott and colleagues have showed that there are also subtle, apparently acquired differences in genetic instability occurring in histologically normal-appearing breast tissue. In these ingenious experiments, they found patches of normal epithelial cells exhibiting hypermethylation of the p16INK4a promoter, leading to overexpression of COX-2 (a well-known carcinogenic
factor) and telomere dysfunction, resulting in genetic instability (38–40). Thus, as the investigators suggested, evaluating levels of COX-2 may be particularly useful in determining risk in benign breast biopsies. There are potentially many ways to quantify the degree of molecular heterogeneity and relative genetic instability in premalignant breast lesions—for example, by measuring DNA repair activity as a surrogate of genetic instability in biopsies of benign breast tissue, which should be explored as potentially powerful biomarkers of risk.

Most of this discussion has focused on short-term strategies to promote molecular prevention research, emphasizing biomarkers of risk. Other molecular strategies which could be even more useful, but whose development will take more time, include discovering diagnostic biomarkers that are specific for certain premalignant lesions, such as ADH or DCIS, which could lead to targeted diagnostic imaging, enabling surgeons to completely excise them. Because more than 90% of all premalignant breast lesions express a very high level of ER, perhaps simply measuring the density of ER by targeted imaging would help determine risk, a molecular analogy to mammographic breast density. A comprehensive understanding of molecular alterations responsible for the evolution of normal cells and premalignant lesions to breast cancer may eventually even lead to safe, easily tolerated, and effective targeted approaches for breast cancer prevention (11).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References


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