# The changing face of tumor phenotypes

"*Determining whether a breast cancer is new or recurrent has important implications, especially in the face of metastatic disease.*"

**KEYWORDS: biomarker expression change ■ intratumoral heterogeneity ■ metastatic cancer ■ recurrent cancer ■ tumor phenotype**

**Robyn Macfarlane**

*Dalhousie University, Halifax, NS, Canada*

**Edward R Sauter**

*Author for correspondence:*
*Cancer Treatment & Prevention Center, University of Texas Health Science Center, Tyler, TX 75707, USA*
*edward.sauter@uthct.edu*

Being diagnosed with cancer is a traumatic event. While a multiplicity of factors contribute to this trauma, uncertainty is one of the most significant. Uncertainty pertaining to the types and tolerance of treatment; uncertainty with regard to the outcome and follow up; and uncertainty pertaining to prognosis are all major factors. After running the gauntlet of diagnosis, staging and attempted curative treatment for a localized malignancy, being diagnosed with recurrent or metastatic disease is indeed devastating. With few exceptions, metastatic solid-organ cancer is incurable. Having said that, metastatic cancer can behave in an extremely heterogeneous manner, making prognostication for patients challenging.

For individuals who have been diagnosed with cancer in the past, treated, and declared disease free, the diagnosis of cancer with histologic features indicative of the same organ of origin raises an immediate question: is this tumor new or recurrent? In this article, we will use breast cancer as a representative tumor type, but this concept applies to other malignancies as well. The determination of whether a breast cancer is new or recurrent depends on whether the newly diagnosed malignancy is in the organ of origin, and locally or regionally metastatic. For newly diagnosed malignancies identified in the same location as the previously treated lesion, it is generally believed to be recurrent. On the other hand, if the new tumor is in a different location, the conclusion is less certain, especially for tumors such as those in the breast, which can be multifocal and/or multicentric. For a lesion identified outside the breast, one presumes it to be a recurrence/persistence of disease rather than a new cancer. Nonetheless, it is well described that some women present with regional or distant spread of breast cancer in the absence of an identified primary [1].

Determining whether a breast cancer is new or recurrent has important implications, especially in the face of metastatic disease. Prognostication for women newly diagnosed with metastatic breast cancer depends on a number of clinical and immunohistochemical parameters. Time from primary disease to relapse, distribution of metastatic disease and performance status are all essential factors to consider when predicting median survival for patients. Equally important, and some would argue more important, are the hormone receptor and HER2 status of the primary lesion. Historically, recurrent or metastatic breast cancer was thought to have the same molecular phenotype as the original lesion, and treatment options have been based upon this assumption. In recent years, there have been a number of retrospective and small prospective studies that have cast doubt on the well-entrenched idea that metastases have the same molecular phenotype (i.e., ER, PR and HER2 status) as the original tumor. The question is, why?

## Biopsy type & size may influence recorded biomarker expression

As mentioned above, clinicians rely heavily on the characteristics of the primary tumor when anticipating risk of relapse and determining treatment options and predicting prognosis in the metastatic setting. Often when women are diagnosed with breast cancer, immunohistochemical analysis of ER, PR and HER2 are performed on the core biopsy rather than the surgical specimen, but there is evidence that significant intratumoral heterogeneity exists. Rates of heterogeneity within a tumor range from 5 to 30% [2] for HER2, but are thought to be somewhat less for ER [3].

The issue of intratumoral heterogeneity is not limited to ER/PR or HER2 receptor status, however; heterogeneity is also demonstrated in the context of markers of proliferation. Investigators

Future Medicine part of fsg

in Sweden evaluated potential differences in proliferation scores between core biopsies and surgical samples among patients who have not received intervening anticancer treatment [4]. They assessed 50 consecutive breast cancer cases by immunohistochemical expression of Ki67 with both a core biopsy and a surgical sample available, without intervening neoadjuvant therapy. Two hundred tumor cells showed an absolute average proliferation difference of 3.9% between core biopsies and surgical samples ($p = 0.046$), with the core biopsies being more proliferative. A corresponding analysis on a log-adjusted scale showed the average relative decrease from the biopsy to the surgical specimen to be 19% ($p = 0.029$). Twelve of the 50 sample pairs had at least 20% discrepant proliferation status, and ten showed high Ki67 in the core biopsy compared with two in the surgical specimen ($p = 0.039$, McNemar's test). On the other hand, comparison samples were not significantly different between core and surgical specimens when 1000 tumor cells were evaluated. Comparing proliferation values for the initial 200 versus the final 800 cancer cells showed significant absolute differences for both core biopsies and surgical samples of 5.3 and 3.2%, respectively ($p < 0.0001$, paired t-test). The authors propose that the reason for the higher proliferation in the first 200 tumor cells sampled than in the next 800 is that the investigators likely focused on areas of higher proliferation initially (i.e., 'hot spots'). Hot spots were either lacking or fewer in number in the next 800 tumor cells compared with the first 200.

## Treatment can change tumor marker expression

It is known that tumor treatment can change tumor phenotype. A recent report of neoadjuvant endocrine therapy in postmenopausal women with locally advanced $ER^+$ breast cancers found that the selective ER downregulator fulvestrant decreased the Ki67 labeling index and ER (but not PR) expression after 4 weeks, and decreased all three markers after 16 weeks of treatment in a dose-dependent fashion [5].

A total of 209 women treated with neoadjuvant chemotherapy after being diagnosed with breast cancer were evaluated for changes in tumor grade, as well as ER, PR and HER2. After neoadjuvant chemotherapy, the pathologic appearance and grade changed in 6.8 and 34.9% of the cases, respectively, while ER, PR and HER2 expression changed by 42.4, 55.4 and 26.6%, respectively. Therefore, the authors concluded that pathologic appearance, grade, ER, PR and HER2 should be re-evaluated after neoadjuvant chemotherapy [6]. This adds to a growing body of evidence that calls into question the well-entrenched practice of relying on the ER, PR and HER2 status of the primary tumor when developing a treatment plan for women who have relapsed or developed metastatic disease. If the pathologic appearance, grade, ER, PR and HER2 status can be altered immediately after neoadjuvant therapy, it is possible, perhaps even probable, that metastatic disease occurring after chemotherapy and/or hormonal therapy and/or HER2-targeted therapy and/or radiation therapy and/or time may fail to be appropriately represented by the original tumor's molecular phenotype.

## Gene expression can change with either local or distant tumor relapse

Given the issues of intratumoral heterogeneity and the potential for treatment to alter tumor marker expression, it is unsurprising that a number of studies have recently shown discordance in ER/PR and HER2 status between the initial breast cancer and the relapsed or metastatic lesion.

Macfarlane and colleagues retrospectively assessed the molecular phenotype of the original tumor (which was included in a large tissue microarray stained with modern techniques) and the biopsy-proven metastatic tumor (stained in the same manner as the original tumor) of 160 patients with relapsed breast cancer [7]. It was demonstrated that there was a 19.4% rate of discordance in the ER/PR or HER2 status between the primary and relapsed lesion in the context of either regional or distant metastases. A total of 5% of tumors had a receptor status change from $ER^+/PR^+$ to $ER^-/PR^-$, and 9.4% went from $ER^-/PR^-$ to $ER^+/PR^+$. For HER2, 3.8% of tumors went from positive to negative and 1.3% went from negative to positive. In this study, every attempt was made to account for the possibility of new breast cancer by excluding patients with in-breast recurrences or a contralateral breast cancer.

> *"Given the issues of intratumoral heterogeneity and the potential for treatment to alter tumor marker expression, it is unsurprising that a number of studies have recently shown discordance in ER/PR and HER2 status between the initial breast cancer and the relapsed or metastatic lesion."*

Other series have demonstrated similar rates of discordance between the primary and metastatic breast cancer lesions with respect to molecular phenotype. Most recently, Lindstrom and colleagues

retrospectively evaluated a large cohort of Swedish patients who had biopsy-proven relapsed/metastatic disease [8]. Rates of discordance for ER, PR and HER2 were 32.4, 40.7 and 14.5%, respectively. Interestingly, some patients had multiple biopsies at various time points along the trajectory of their disease with variable hormonal and HER2 results, raising the question of heterogeneity not only within a specific tumor deposit, but between various metastatic sites. Importantly, there was a statistically significant difference in overall survival in favor of women with stable $ER^+$ tumors versus those who went from $ER^+$ to $ER^-$ at relapse (hazard ratio: 1.48; 95% CI: 1.08–2.05) in this study [8].

There are certainly limitations with retrospective series and, thus, a number of investigators have attempted to confirm the apparent change in molecular phenotype from the diagnosis of localized disease to recurrent/metastatic disease in a prospective manner. Amir and colleagues conducted a single-arm prospective study mandating biopsy at the time of relapse [9]. Although the investigators reported a 38.8% rate of discordance in ER, PR and/or HER2 (which would result in a significant treatment change in 15.9%), a number of cases were ipsilateral breast recurrences, raising the question of whether these represented new primary breast cancers. Other prospective studies are ongoing.

The rates of discordance in hormonal and HER2 status between primary and metastatic breast cancer have been consistently reported in both retrospective and prospective series to be at least 20%. The question we now have to entertain is: why? As outlined above, issues relating to tumor heterogeneity may play a role. Perhaps what we have been observing is not a true change in molecular phenotype, but is rather a consequence of undersampling the primary tumor. Treatment-induced changes have also been presented as a possible explanation for observed discordance. However, none of the published retrospective series were able to detect a significant pattern of change that correlated with a specific adjuvant treatment, but the caveat to this is that the numbers being assessed were universally small. An issue of concern in the context of a retrospective series is the potential loss of antigen over time in formalin-fixed paraffin-embedded tissue. Although this could account for changes in hormonal status going from positive to negative, it cannot account for the reverse. The survival of a stem cell that can differentiate over time is also an appealing explanation; however, more research is needed to confirm/refute this possibility.

> *"…as clinicians we are faced with a growing body of evidence suggesting that there may be discordance in the molecular phenotype between the primary and the relapsed breast cancer lesion. Many uncertainties remain with regard to how to contextualize this information."*

As clinicians we are faced with a growing body of evidence suggesting that there may be discordance in the molecular phenotype between the primary and the relapsed breast cancer lesion. Many uncertainties remain with regard to how to contextualize this information. Should all patients be rebiopsied at the time of diagnosis with metastatic disease? If there is a change in the hormonal and/or HER2 status, should treatment decisions in the metastatic setting proceed based upon the results from the second biopsy? What do we tell our patients if there is a change in receptor status with respect to prognostication? Is there a role for a prospective, randomized study mandating biopsy? What noninvasive biomarkers may contribute to this clinical dilemma?

**Financial & competing interests disclosure**

*The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.*

*No writing assistance was utilized in the production of this manuscript.*

## References

1 Pentheroudakis G, Lazaridis G, Pavlidis N. Axillary nodal metastases from carcinoma of unknown primary (CUPAx): a systematic review of published evidence. *Breast Cancer Res. Treat.* 119(1), 1–11 (2010).

2 Vance GH, Barry TS, Bloom KJ *et al.* Genetic heterogeneity in HER2 testing in breast cancer: panel summary and guidelines. *Arch. Pathol. Lab. Med.* 133(4), 611–612 (2009).

3 Layfield LJ, Saria E, Mooney EE, Liu K, Dodge RR. Tissue heterogeneity of immunohistochemically detected estrogen receptor. Implications for image analysis quantification. *Am. J. Clin. Pathol.* 110(6), 758–764 (1998).

4 Romero Q, Bendahl PO, Klintman M *et al.* Ki67 proliferation in core biopsies versus surgical samples – a model for neo-adjuvant breast cancer studies. *BMC Cancer* 11, 341 (2011).

5 Kuter I, Gee JM, Hegg R *et al.* Dose-dependent change in biomarkers during neoadjuvant endocrine therapy with fulvestrant: results from NEWEST, a

randomized Phase II study. *Breast Cancer Res. Treat.* 133, 237–246 (2012).

6 Zheng S, Zhang BL, Xiao T *et al.* [Comparison of histopathologic changes and expression of biomarkers in breast carcinoma before and after neoadjuvant chemotherapy.] *Zhonghua Bing Li Xue Za Zhi* 40, 465–470 (2011).

7 Macfarlane R, Seal M, Speers C *et al.* Molecular alterations between the primary breast cancer and thesubsequent locoregional/metastatic tumor. *Oncologist* 17, 172–178 (2012).

8 Lindstrom LS, Karlsson E, Wilking UM *et al.* Clinically used breast cancer markers such as estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 are unstable throughout tumor progression. *J. Clin. Oncol.* 30(21), 2601–2608 (2012).

9 Amir E, Clemons M, Freedman OC *et al.* Tissue confirmation of disease recurrence in patients with breast cancer: pooled analysis of two large prospective studies. *J. Clin. Oncol.* 28(Suppl. 15), Abstract 1007 (2010).