Gene signatures of genomic instability as prognostic tools for breast cancer

“...multigene signatures have emerged as an important molecular tool that can provide useful prognostic and predictive information...”

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Breast cancer remains a challenge to treat, in part owing to the heterogeneity of the disease. Breast cancers vary greatly in terms of prognosis and in the way they respond to targeted or systemic therapies [1,2]. Unfortunately, chemotherapy is associated with short- and long-term toxicities [3]. Therefore, it is crucial to identify patients who can be spared from unnecessary adjuvant therapy and those who must be treated with aggressive therapies. In the last decade, multigene signatures have emerged as an important molecular tool that can provide useful prognostic and predictive information in addition to classical clinicopathological criteria (reviewed in [4,5]). The first expression profiling studies in breast cancers have succeeded in defining molecular subtypes with distinct prognosis: luminal epithelial/estrogen receptor-positive (ER⁺) (luminal A, luminal B), HER2⁺, basal-like and normal-like [6–8]. Subsequent expression profiling studies have been performed with the aim of defining a relatively small set of genes whose expression at the mRNA level would help identify breast cancer patients who are at risk of recurrence (reviewed in [4,5,9]). Most gene signatures include a large component of genes that are involved in cell proliferation [10–16]. Interestingly, these signatures appear to track similar biological signatures as the major subtypes [17–19]. Other gene signatures have focused on distinct biological processes such as wound response [20,21], invasiveness [22], ‘stemness’ [23–25] or genetic instability [26–28].

Genomic instability, as defined by the stemline scatter index [29], was analyzed in 890 primary breast carcinoma patients and was shown to be a useful prognostic indicator, independent of established clinical factors [26]. Comparing

genomic instability and expression profiling data, Habermann and coworkers defined a 12-gene genomic instability signature that was shown to predict metastasis-free survival in three independent breast cancer datasets, and represented a prognostic marker that is independent of traditional clinicopathologic factors [27]. Interestingly, a firm association was established between genomic instability and breast cancer subtypes [27].

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In a study by Carter and coworkers, a signature of chromosomal instability was established from specific genes whose expression was consistently correlated with chromosomal aberrations [28]. The rationale was to use gene expression data to infer chromosomal imbalance at sub-band resolution. Therefore, the authors did not attempt to identify genes that cause chromosomal instability (CIN), but instead derived lists of 70 or 25 genes, CIN70 and CIN25, respectively, whose differential expression was symptomatic of chromosomal instability. The CIN25 signature was shown to be a significant predictor of clinical outcome in 12 out of 18 tumor datasets, including four breast tumor databases [28]. Another prognostic signature, termed complexity index in sarcoma (CINSARC), was established by combining genes that were differentially expressed in 183 sarcomas when tumors were classified using either comparative genomic

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hybridization array analysis, histological grade or the CIN70 signature [30]. All these genes were submitted to gene ontology analysis to keep 67 genes that belonged to overrepresented pathways for comparative genomic hybridization or histological grade. A training/validation methodology was then used to demonstrate that the CINSARC signature was able to predict outcome in two breast cancer databases [12,13].

In a recent study on the mechanism by which the CUX1 homeodomain protein promotes genetic instability, we have uncovered a gene signature that can predict clinical outcome among ER⁺/lymph node-negative (LN⁻) breast cancer patients [31]. CUX1 is a transcription factor that is overexpressed in various human cancers [32–34], particularly in breast cancers [35,36]. *In situ* hybridization performed on multiple tissue core arrays showed increased *CUX1* expression within high-grade, but not low-grade, breast carcinomas [36]. Immunohistochemical analysis on a separate series of breast and pancreatic cancer tissues also demonstrated that CUX1 protein expression was increased in the high histological-grade tumors compared with low-grade tumors [36,37]. Moreover, among patients with grade 3 breast tumors, *CUX1* mRNA expression inversely correlated with relapse-free and overall survival [36]. In tissue culture systems, CUX1 was shown to accelerate entry into S phase during cell-cycle progression, and to promote cell motility and invasion [36–39]. In mouse models, expression of CUX1 in the mammary gland of transgenic mice causes the development of mammary tumors of various histological types that exhibit extensive genetic instability [31,40]. Recent work led to the identification and validation of CUX1 transcriptional targets that promote DNA replication, prevent multipolar division and enable the survival of cells that contain more than two centrosomes [31]. These cells evolved to become aneuploid and tumorigenic [31]. CUX1 targets a defined 29-gene expression signature termed CUX1 signature (C1S), which was then applied to 12 previously published gene expression datasets derived from whole-tumor breast tissues [31]. The breast cancer molecular subtypes were not distributed equally between the ‘low’- and ‘high’-expression groups as classified by the C1S [31,41]. Those subtypes with increased genetic instability and a poorer outcome, namely HER2⁺ and basal-like [27,42], were over-represented in the C1S high-expression group, while the proportion in the good outcome-enriched luminal A subtype was much reduced (see Figure 4 in [31]). However, the signature identified subgroups of ER⁺/LN⁻ patients with

poorer clinical outcome [31]. Furthermore, when this cohort was separated into subtypes, the signature was able to identify poor outcome among the luminal A group, which is the subtype considered to be at the lowest risk [31]. Importantly, multivariate COX regression analysis demonstrated that the C1S signature is a significant predictor of outcome that is independent of grade, LN and ER status [31].

We contend that gene lists that track down genetic instability have the potential to greatly improve the prognostic value of gene signatures. Indeed, a crucial property associated with tumor progression is the ability of tumor cells to evolve rapidly and generate large repertoires of genetic variants. Most genetic variations are expected to be deleterious; however, if genetic variants reach a high number or are generated at a high enough frequency, rare variants will unavoidably emerge with increased proliferative, invasive and immune-suppressive properties. The capacity of tumor cells to evolve is evidenced in the biological heterogeneity of tumors [43–45], as well as in their genomic heterogeneity [46–49]. Moreover, the fact that metastatic tumor cells display an expression profile and exhibit biological properties that are different from that of the primary tumor has been amply documented in several studies [50–53]. If we accept, as a premise, that primary tumor cells must evolve in order to acquire novel metastatic properties, it follows logically that a gene signature that measures the evolutionary potential of primary tumors will also predict clinical outcome.

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Interestingly, the CINSARC and C1S signatures have much in common [30,31]. A total of 14 out of the 29 genes in the C1S list are present within CINSARC. Moreover, genes in both signatures are involved in the same biological processes, which can be summarized as gene replication, mitosis checkpoint and centrosome dynamics. The similarities in cellular functions between the C1S and CINSARC signatures are interesting in that they shed light on the underlying mechanisms of genetic instability in cancer cells and confirm the generality of findings made in each of these studies. While tetraploidy

is observed in only a small minority of breast cancers [54], numerical and structural centrosome aberrations can be detected in a sizeable fraction of cells in breast lesion biopsies [29,55]. Importantly, a clear association was established between centrosome aberrations and genetic instability [55,56]. The mechanistic link between genetic instability and structural abnormalities in centrosomes remains to be defined; however, recent evidence has shed light on the consequences of having extra centrosome numbers [57]. In experimental systems, centrosome amplification was shown to cause the formation of multipolar spindles in mitosis. In normal cells, multipolar divisions follow the formation of multipolar spindles and generate mostly nonviable daughter cells. By contrast, certain cancer cells, notably cells that express the C1S signature, are able to engage a robust spindle assembly checkpoint that extends the duration of mitosis until the multiple centrosomes have clustered into two poles, thereby allowing bipolar mitosis and cell survival [31,58]. Yet, transient multipolar spindles in these cells are associated with increased occurrence of merotelic, which, if not corrected, causes chromosome missegregation [57,59]. Other genes in the

C1S signatures are directly involved in centrosome dynamics and may contribute to chromosomal instability by mechanisms that remain to be defined [31]. Overall, the information obtained from multiple studies suggest that protection from mitotic catastrophe together with frequent chromosome missegregation events contribute to the generation of large populations of genetic variants from which tumorigenic cells emerge with metastatic properties. These findings not only increase our understanding of the molecular mechanisms underlying genomic instability, they also provide gene signatures that will serve as a useful prognostic tool for lymph node-negative estrogen receptor-positive breast cancers.

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