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INVITED COMMENTARY

# Struggling with subtypes: trying to bridge the gap between molecular breast cancer subtypes and clinical management

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Modern understanding of breast cancer as a disease has undisputedly left the "Halstedian" worldview and has entered the molecular age. Formerly, breast cancer heterogeneity was defined simply by measurable differences such as nodal involvement or tumor size and subsequently by steroid hormone receptor positivity. Yet, the groundbreaking discoveries of Perou et al. [1] have taught us that there are distinct molecular subtypes of breast cancer that even carry clinical relevance with regard to patient outcome [2]. The fact that these subtypes and their clinical impact on breast cancer outcome can be consistently identified across data sets strongly suggests that they are indeed indicative of distinct intrinsic biological properties and behavior [3].

By far the most frequent molecular subtypes are luminal tumors, encompassing about two-thirds of all breast cancers. For luminal tumors as a whole, the molecular characterization seems to be rather concordant with the immunohistochemical luminal definition (ER positive, HER2 negative) [4]. Yet, further sub-classification between luminal A and B is not yet well defined, even though there seems to be a clear difference in outcome [3]. Whereas luminal A tumors and basal-like tumors differ substantially not only in immunohistochemical criteria but also—as reliably analyzed by several gene array platforms—in molecular properties [5], luminal A and B tumors both express steroid hormone receptors. Thus, they are currently subject to at least overlapping therapeutic approaches. So far, the most commonly used discriminators between luminal A and B tumors are proliferation genes or signatures such as the genomic grade index [6]. By simple means, luminal A and B subtypes can also be reproducibly distinguished by proliferative activity, as demonstrated by Cheang et al. [7], who optimized a Ki-67 cut-off of 13.25% and validated its clinical significance in a large, independent data set. However, on a molecular level, the distinction between luminal A and B is not as clear-cut [3].

Karn et al. [8] in their elegant study sought to identify genes that are differentially expressed between luminal A and luminal B subtypes and that are not associated with proliferation. Their underlying hypothesis was that there are distinct molecular characteristics unique to either of these subtypes and that their discovery may bear the potential for therapeutic implications.

In a discovery set of 171 early breast cancers from their own center, the authors used Affymetrix arrays to discover genes unique to luminal B breast cancer but not to luminal A or highly proliferating triple-negative tumors. Their discovery yielded a gene set of 18 genes, most without any prior connection to breast cancer. One of the genes identified was the scaffold protein sodium exchanger regulatory factor (NHERF1). NHERF1 transcription is regulated by estrogen, and its protein expression and also subcellular distribution seem to be associated with breast tumor progression [9]. NHERF1 also co-localizes with HER2 in HER2-overexpressing tumors, thus suggesting a role in HER2-mediated signal transduction [10].

Karn et al. [8] validated their findings in a large, independent dataset ($n = 3030$) consisting of less than 10% of their own samples and 2792 samples from publicly available data sets. In accordance with the literature, NHERF1 expression correlated positively with ER expression and

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HER2 status as well as with lymph node status, a surrogate for tumor progression. In ER positive but not ER negative disease, high NHERF1 expression was significantly correlated with poor outcome, suggesting a potential predictive component regarding response to endocrine therapy. The authors consequently analyzed NHERF1 regarding patient outcome in homogeneous subgroups according to adjuvant therapy. In low-NHERF1 tumors, patients who had received adjuvant endocrine therapy did have a significantly better outcome than those who had not received adjuvant systemic therapy. In high-NHERF1 tumors, no such difference was seen. Even though these univariate analyses may help to generate hypotheses regarding a predictive value, they are by no means a definite proof, since they do not correct for underlying biases by clinicians or patients regarding their decision for or against a certain treatment. Unfortunately, a multivariate model with interaction terms which would have helped to account for such biases is not provided. In multivariate analysis in ER positive tumors, accounting only for clinico-pathological factors, NHERF1, together with tumor size, was a significant factor for disease recurrence.

The study of Karn et al. [8] has certainly considerable strengths but also limitations, like most retrospective studies looking at prognostic and predictive information in heterogeneous patient collectives. Important strengths of this study include the use of an initial discovery cohort and the consecutive validation in an independent substantial cohort derived from large published data sets. However, the use of different heterogeneous patient collectives with different treatment regimens may also pose a difficulty in interpretation, particularly when essential information such as treatment is not controlled for in multivariate analysis. Nevertheless, the clinical associations found by the authors are in accordance with prior information.

Most importantly, the authors set out to find molecular differences between luminal A and B tumors that are not reflected in proliferative activity. Yet, their candidate marker NHERF1 lost its prognostic impact in multivariate analysis when Ki-67 expression was added as a variable. Thus, the article cannot convincingly answer the initial question about the driving forces in luminal B breast cancer—whether it is actually just proliferation or other more fundamental pathways. One hint toward a more fundamental biological difference in the luminal subtypes is the observation that the predictive impact on endocrine response observed for NHERF1 could not be seen for Ki-67 alone. Last but not least, it would have been of interest to also see the clinical impact of the other 17 genes that were differentially expressed between luminal A and B tumors.

In conclusion, even though we have finally conceptually accepted that breast cancer consists of at least four molecular subtypes, we have not yet translated this knowledge into clinical therapy strategies. New assays suitable for paraffin-embedded tissues such as PAMM50 [11] may enable us to assess these subtypes on a routine basis. Nonetheless, up to now solely ER, PR, and HER2 are used as predictive markers in early breast cancer to select the type of adjuvant therapy. In addition, risk assessment based on invasion markers uPA/PAI-1 [12, 13] or gene signatures such as the recurrence score [14] or the 70 gene signature [15] is used in early hormone receptor positive (i.e., luminal) tumors for the decision whether or not to add chemotherapy to endocrine therapy. All of these prognostic assays also carry a certain predictive component regarding increased response to adjuvant chemotherapy in high-risk patients. Thus, inadvertently, they also measure certain properties that actually distinguish luminal A from luminal B tumors. Yet, in modern breast cancer management, we should aim to target tumor biology and not mere relapse risk. Karn et al. [8] show us a way how to get beyond descriptional biology regarding luminal B tumors and suggest a potential candidate for therapeutic interventions. Whether NHERF1 in particular has the potential of becoming clinically relevant either as a predictive factor or as a target for interventions, e.g., with the epidermal growth factor receptor pathway, will depend mainly on future pre-clinical evaluations regarding its functional properties. Its ability to bind to growth factor receptors such as EGF-R and thus increase signaling [16]—as the author suggests—a correlation with the PI3K/Akt pathway, make it an obvious target at least for further investigations.

The immediate clinical usefulness of the findings by Karn et al. may still be rather limited, but their article certainly contributes to the functional understanding of luminal B breast cancer. In the future, we should aim to deepen this functional understanding but at the same time work hard on translating it into immediate therapeutic consequences. We, thus, urgently need potentially practice-changing clinical trials in early breast cancer that tailor therapy approaches to luminal A versus B tumors. Ultimately, however, we would be ill-advised to throw out the baby with the bath water. It is not (yet) time to pack up the pathologist's microscope [17]. For clinical utility, integration of genomic data with clinico-pathological variables are likely to render optimal results [18]—thereby somewhat reconciling Halsted with the molecular age.

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