Letter

# Are triple-negative tumours and basal-like breast cancer synonymous?

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Kreike and colleagues [1] examined the gene expression and pathological characteristics of a retrospectively accrued cohort of 97 triple-negative phenotype (TNP) (oestrogen receptor [ER]-negative, progesterone receptor-negative, and HER2-negative) invasive breast cancers. TNP tumours were profiled with oligonucleotide microarrays and compared with a control group of 102 non-TNP tumours, which were obtained from an unrelated project. The authors then investigated whether the TNP would accurately identify basal-like cancers, by assessing the correlation coefficient between the gene expression profiles of each TNP cancer and the centroids of the molecular subgroups as defined by Hu and colleagues [2]. As expected, the majority (91%) of TNP tumours were classified as 'basal-like' tumours. However, 9% of tumours either showed a normal-like phenotype or were unclassifiable [1]. The authors presented a hierarchical clustering analysis of both TNP and control cases, based on a partial 'intrinsic gene list' and a different reference RNA when compared with those reported by Hu and colleagues [2], and observed that all of the TNP group and 18.6% of the control non-TNP group clustered together [1]. Based on the above results, the authors drew the provocative conclusions that 'basal-like tumours can be reliably defined by triple-negative immunohistochemistry' and that 'triple-negative tumours are synonymous with basal-like tumours'.

We believe that equating TNP tumours with basal-like breast cancer is misleading [3] and, in fact, is not supported by the data the authors themselves present. Given that only 91% of TNP tumours displayed a significant association with the basal-like centroid and that 18.6% of non-TNP tumours clustered together with TNP tumours in the 'basal-like' cluster, a more reasonable conclusion is that the majority of, but not all, TNP tumours have a basal-like phenotype and that the majority of, but not all, basal-like tumours have a TNP phenotype. Therefore, it is also reasonable to conclude that the above findings do not demonstrate that TNP tumours are synonymous with basal-like tumours.

One could argue that the results of the study conducted by Kreike and colleagues [1] are, in fact, in almost complete agreement with those of previous studies that demonstrate that a TNP immunohistochemical (IHC) phenotype is not an ideal surrogate for the identification of microarray-defined basal-like breast cancers [4-9]. Based on previous expression profiling/hierarchical clustering analysis, not only basal-like cancers but also normal breast-like cancers harbour a TNP phenotype at the mRNA level [8,10,11]. Importantly, normal breast-like tumours have been shown to have a distinct response to neoadjuvant chemotherapy [8] and prognosis when compared with basal-like breast tumours. The only IHC signature of basal-like breast tumours which has been validated by expression profiling demonstrated that a panel composed of ER, HER2, Ck 5/6, and epidermal growth factor receptor (EGFR) can identify these tumours with 100% specificity and 76% sensitivity [4]. In that study [4], if basal markers (that is, Ck 5/6 and EGFR) were not included, the specificity of the signature (that is, solely composed of ER and HER2) would be significantly reduced with a marginal increase in the sensitivity [4]. Furthermore, apart from the

EGFR = epidermal growth factor receptor; ER = oestrogen receptor; IHC = immunohistochemical; TNP = triple-negative phenotype.

remarkably low prevalence of EGFR expression in TNP tumours, the results of Kreike and colleagues [1] are in agreement with those of several previously published comparisons on the IHC features of basal-like tumours as defined by expression arrays, which clearly demonstrate that most, but certainly not all, have a TNP phenotype [8,11,12]. In fact, ER IHC expression and HER2 3+ or gene amplification are reported to be found in 5% to 45% and 5% to 15% of basal-like tumours as defined by expression arrays, respectively [3,8,9,11,12]. In addition, Harris and colleagues [13] recently reported on a subgroup of *HER2*-amplified breast cancers that harbour a basal-like transcriptomic profile.

Previous studies have shown that the expression of 'basal markers' (that is, Ck 5/6, Ck 14, Ck 17, and/or EGFR) is associated with a poor prognosis [5-7,14], regardless of hormone receptor expression. The expression of basal markers (basal cytokeratins and EGFR) in TNP tumours (core basal phenotype) also correlates with a worse prognosis and identifies a clinically distinct subgroup within the TNP group [5,7]. Moreover, it should be noted that identification of a subgroup of tumours solely based on the lack of expression of immunohistochemistry (for example, TNP) risks misassignment based on technical artifacts [3,4].

From a technical perspective, a word of caution should be voiced concerning the design of the study of Kreike and colleagues [1]: slide batch spotting biases and differences in the reference RNA used have been reported to have a significant effect on microarray data analysis [15]. When these biases are not corrected by additional processing and rescaling of the data [15-17] and cases are subjected to hierarchical clustering analysis, cluster assignment is typically biased by the noise inherent in each slide batch and/or reference. Hence, it is not clear to what extent the 97 TNP samples in this study clustered together owing purely to the similarities of their gene expression biology as opposed to the contributions to the expression profiles induced by distinct slide batches and/or references used for the analysis of TNP and control groups (so-called 'batch effect') [15-18].

Molecular profiling undoubtedly has had a dramatic impact on our understanding of breast cancer [4,10,11]. Given the difficulties in applying expression array analysis to identify the molecular subgroups in clinical practice (in particular, when formalin-fixed paraffin-embedded samples are used), the identification of simple IHC panels that reliably identify these subgroups, as described by Kreike and colleagues [1], is undeniably a meritorious effort. However, caution should be exercised in the translation of results obtained with mRNA-based expression analysis to IHC markers. Two of the most pressing challenges of breast cancer research are (a) to unravel the complexity of TNP tumours and basal-like breast carcinomas and (b) to identify novel therapeutic targets for these tumours. Blurring the boundaries of these two subgroups of breast tumours by using surrogate markers derived from microarray-based studies that are not optimally designed may lead to misleading conclusions and serve only to further confound the study of already enigmatic and clinically challenging entities.

## Competing interests

The authors declare that they have no competing interests.

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