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Letter to the Editor

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To the editor: Lerma *et al*[1] have reported on the immunohistochemical heterogeneity of breast carcinomas with the triple-negative (oestrogen receptor (ER)-negative, progesterone receptor-negative and HER2-negative) phenotype using 23 biomarkers. The authors have claimed that triple-negative and basal-like tumours are one entity and that triple-negative phenotype could be used as a surrogate for basal-like cancer. We agree with the authors in that a better understanding of the biology of triple-negative cancers is of paramount importance for the identification of ideal systemic therapy regimens and novel therapeutic targets for these tumours. However, we believe that equating triple-negative tumours with basal-like breast cancer is misleading. There are several lines of evidence that triple-negative phenotype is not an ideal surrogate for identification of basal-like breast cancers. Most authorities regard microarray-based expression profiling analysis as the 'gold standard' for identification of basal-like breast cancer. Although majority of basal-like cancer, as identified by this method, lack hormone receptors and HER2 expression, ER immunohistochemical expression and HER2 3+ or gene amplification are reported to be found in 5–45 and 5–15% of basal-like cancers, respectively.[2–4]

On the basis of dendrograms of expression profiling/hierarchical clustering analysis, the group showing a triple-negative phenotype at the mRNA level encompasses at least two subgroups, basal-like and normal breast like cancers. Although the latter group is still poorly characterised, they are reported to have a prognosis that seems to be better than that of basal-like cancers,[4,5] and do not seem to respond to neoadjuvant chemotherapy.[3] We[6,7] and others[8] have demonstrated that the expression of basal markers (ie, basal cytokeratins and EGFR) identifies a clinically significant subgroup within the triple-negative group. On the other hand, expression of basal cytokeratins and/or EGFR,[7,8] regardless of the expression of hormone receptors status, identifies a subgroup of cancers persistently showing poor prognosis.

Another caveat that needs to be voiced is the identification of a subgroup of tumours solely based on the lack of expression of three immunohistochemical markers. As stressed by Nielsen *et al*[9], 'lack of staining for ER and HER2 alone to identify basal-like breast cancers risks mis-assignment based on technical failures'.

In addition, Lerma *et al* have subdivided triple-negative tumours into two subtypes based on the expression of myoepithelium (ME)-specific markers (pure basal variant and myoepithelial variant, which express SMA and/or S100). Although this approach is of interest, this classification needs further consideration. It is currently accepted that both triple-negative and basal-like tumours are heterogeneous groups that include tumours with favourable prognosis, such as medullary-like and salivary gland-like cancer.[10] Expression of these ME markers (S100 and SMA) is not entirely restricted to triple-negative tumours. The classification of S100- and SMA-negative triple-negative tumours as pure basal-like cancer may be misleading, if the expression of other previously validated 'basal' markers (basal cytokeratins and EGFR) and myoepithelial markers is not assessed. If triple-negative tumours were classified into pure basal and ME subtypes solely based on the expression of SMA and S100, there are tumours that are negative for those two markers, but express other markers preferentially found in myoepithelial cells, such as p63, 14-3-3sigma, maspin, smooth-muscle myosin heavy chain, CD10 and caveolin 1.[11] For instance, it would be rather disputable to classify as pure basal-like cancers those triple-negative tumours lacking S100 and SMA and expressing p63 and smooth-muscle myosin heavy chain. In a previous publication,[12] we have demonstrated that tumours, which express both basal cytokeratins and ME markers, are associated with the worst prognosis compared with pure basal cytokeratins expressing tumours or pure ME markers expressing tumours, which showed the best outcome of the three groups. These results show that considering expression of ME markers regardless of the expression of other basal markers may be misleading. Caution should be exercised when translating the mRNA expression profiling-based molecular taxonomy into classes identified by immunohistochemistry alone.

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In reply: In reply to the Letter to the Editor by Drs Rakha, Ellis and Reis-Filho, regarding our article,[1] we would like to clarify the following:

Triple-negative (ER/PR/HER2-negative) breast cancer constitutes more than one entity with heterogeneous and poorly understood growth and invasive mechanisms. Despite concordance between histopathology, immunohistochemistry and cDNA analysis, a pragmatic approach should be considered for identification of these tumors for treatment.[2] We agree that negativity for ER/PR/HER2 is not an obligated criteria to classify breast tumors as basal-like carcinomas, but for the purpose of our study, we only selected the triple-negatives tumors for analyzing several biological markers. The use of both terms indistinctly throughout the text might have led to some confusion.

From a histopathological point of view, it is true that basal-like breast carcinomas are heterogeneous groups of high-grade invasive ductal neoplasms (NOS), which also include most of the myoepithelial, medullary, adenoid cystic, metaplastic and spindle cell carcinomas.[3] Apparently, they are not specific markers to classify these tumors. Recently, it has been stated that the immunohistochemical pattern that best defines basal-like breast carcinomas is the triple negativity in association with the positivity for EGFR and/or basal cytokeratins (CKs) (ie, CK5/6, CK14 and CK17).[4] Few investigators have analyzed all three CKs together, as we did. Nevertheless, in our cases, we did not find any statistical correlation between expression of several CKs (data not included in our paper). Furthermore, how basal CK expression contributes to the adverse prognosis of these tumors is currently unknown. Among our triple-negative cases with myoepithelial differentiation, we also observed a heterogeneous pattern of CK expression. Moreover, positive S100 expression was observed more frequently than SMA expression, and interestingly, in other studies, SMA expression was detected more frequently than p63.[3] It means that the characterization of these neoplasms is still blurry.

The level of EGFR expression has been revealed as an independent predictor of tumor response to radiation therapy.[5] The contribution of our immunohistochemical study in this field is that, in fact, in triple-negative breast carcinomas, several growth-factor receptors (ie, EGFR, IGF1R, PDGFRá, and so on) are involved, and therefore, they could explain the aggressive clinical behavior in some cases.

Other markers, such as p53 overexpression, have been considered a common marker of basal-like breast carcinoma,[6] but it has also been detected in 11.3% of ER-positive breast carcinomas.[7] Therefore, current evidence is still insufficient to support the routine analysis of p53 in clinical practice.[8]

Dendrograms of expression profiling/hierarchical clustering analysis are not available for most institutions. Of note, comparative studies with different gene sets for breast cancer diagnosis achieved 77–81% agreement for outcome classification,[9] as with immunohistochemistry and conventional histopathology.

In summary, the data reviewed above indicate that characterization and/or classification of these neoplasms is still undergoing investigation. Therefore, more research should be conducted in this field to define the underlying mechanisms, which in turn will provide better understanding of breast neoplasms for more accurate patient treatment.