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Prognostic and predictive factors of breast carcinoma: Beyond hormonal receptors and HER2

*Oscar Nappi**, *Giovanna Carrillo*

Division of Pathology, A. Cardarelli Hospital, Naples, Italy

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ABSTRACT

Over the last years, new molecular prognostic and predictive markers in malignant tumours have been daily proposed by basic researchers; their clinical application have consistently contributed to increase the Pathology Services workload; immunohistochemistry often surrogates other more sophisticated biotechnologies with acceptable results although in several cases molecular techniques look preferable. Breast cancer is a model widely studied and effort is made to improve therapeutic results. Conventional macro-microscopic parameters, evaluation by immunohistochemistry of Ki 67, ER, PR and HER2 status represent consolidated and standardised prognostic factors; ER, PR and HER2 status are also validated predictive markers; for HER2 status, in borderline cases, hybridisation *in situ* technologies, such as FISH, improve the definition of result.

In future, other molecular markers for new targeted therapeutic approaches will be probably consolidated; the more promising of them are VEGF, Topoisomerase-alpha II, PTEN and other members of c-erbB (HER) family such as HER1 (EGFR) and HER 3.

It is widely known that breast cancer is a heterogeneous disease embracing several different phenotypes with consistently different biological characteristics.

In order to plan specific therapies for breast cancer as well as other tumours, the pathologist plays a key role in tight relationship with the oncologist. Recently, new classificative approaches prevailing based on genetic more than morphological and immunohistochemical profiles have been proposed; nevertheless, traditional morphological classification continues to be applied and the status of ER, PR and HER2 (c-erb2) are currently the only prognostic markers validated as predictive to therapy response.[1–4]

Other accepted prognostic markers are represented by traditional morphological findings such as tumour size, grade, lymphovascular invasion, lymph nodes status and Ki 67 (Mib1) evaluation by immunohistochemistry.[5,6]

At present, procedures to carry out, on each breast cancer, Estrogen Receptor alpha (ER), Progesteron Receptor (PR) and HER2 status by immunohistochemistry are universally validated; Fluorescence *in situ* hybridisation (FISH), Chromogenic *in situ* hybridisation (CISH) and Silver *in situ* hybridisation (SISH) being alternative accepted methods.[7,8]

Immunohistochemistry is the standard method for determining receptor status of both ER and PR. According to international guidelines, standard criteria for pre-analytical and analytical phases should be established and rigorously controlled by intra- and interlaboratory quality control programmes. Any level of reactivity has to be reported for both receptors since only tumours with no evidence of reactivity are considered non-responsive to endocrine therapy. It is to remark that the immunoreactivity for ER and PR is only nuclear. A popular score, largely diffused in Pathology laboratories, is the Allred score that, ranging from 0 to 5, evaluates the intensity of immunoreactivity and the proportion of stained nuclei.[9]

The Human epidermal growth factor receptor 2 (HER2) is a member of the epidermal growth factor receptor family. The oncogene HER2 is located on chromosome 17q21; its amplification leads to the overexpression of the transmembrane

* Corresponding author.
E-mail address: oscarnappi@tin.it (O. Nappi).

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protein in about 20–25% of breast cancer and represents an adverse prognostic factor associated with high-grade tumours, lymph node metastases and higher rate of mortality. HER2 status is also a specific predictive marker to response to trastuzumab, a humanised monoclonal antibody that, validated by several wide studies, has demonstrated to improve time to progression and overall survival of breast advanced cancer and, more recently, also to reduce risk of recurrence and mortality in adjuvant setting for early breast cancer. Current largely diffuse protocols imply the detection of HER2 protein expression by immunohistochemistry (several validated antibodies are available); a score graded from 0 to 3+ on the basis of intensity of the chromatic signal and the percentage of positive cell, evaluating only the membrane reactivity (chicken wire pattern) is largely used. Cases from 0 to 1+ are considered negative, score 3+ positive and eligible for trastuzumab therapy. Borderline cases (2+) have to be tested for gene amplification by FISH or (alternatively) CISH or more recently SISH.[10]

On the basis of gene microarrays technique, several subtypes of breast carcinomas have been individuated and specifically a *luminal type* subclassifiable in luminal type A, characterised by the highest expression of ER alpha gene and the best prognosis, luminal B and luminal C with a lower level of expression of ER alpha gene and a poorer prognosis, a *basal cell-like type* characterised by the absence of expression of hormone receptors and HER2 and representing a good number of cases of the so-called 'triple negative immunophenotype' and a HER2 overexpressing type; basal cell-like type and HER2 overexpressing type have the worst prognosis.[11–13]

It is absolutely evident that this scheme is strongly suggestive and, on the basis of current knowledge and available drugs, also the best one to classify breast tumours on the clinical standpoint; great effort is developing also to correlate morphological conventional histotypes with these gene tumour profiles, and some interesting result has been reached.

Moreover, new scenarios are emerging in order to individuate predictive markers for new or integrated therapies; for example, cases showing resistance to trastuzumab therapy have evidenced the need to select other markers.

In the future, promising predictive markers for breast carcinoma are VEGF, *Topoisomerase II-alpha*, PTEN and other members of EGFR family.

VEGF (*Vascular endothelial growth factor*) is the main pro-angiogenic factor that also regulates the permeability of endothelial cells; an overexpression of VEGF in several tumours, including breast cancer, is associated with a poor prognosis and preliminary studies have demonstrated the efficacy of bevacizumab, a recombinant humanised monoclonal antibody developed against VEGF that binds to soluble VEGF, to prevent receptor binding and inhibit endothelial cell proliferation and vessel formation.[14]

Topoisomerase II-alpha (*TOP2A*) gene amplification is a potentially useful predictive marker of responsiveness to anthracycline-containing chemotherapy. This gene is frequently co-amplified with HER2 since it is located in the proximity of HER2 in the 17qchromosome. Many studies have demonstrated that TOP2A amplification represents a useful marker to predict the responsiveness to anthracycline-based therapy, also in breast cancer.[15]

PTEN (Phosphatase and tensin homologue deleted on chromosome 10) is a tumour suppressor gene frequently down-regulated in breast carcinoma. PTEN is probably involved in the mechanisms of trastuzumab resistance, loss of function of PTEN, negative regulator of phosphatidylinositol 3-kinase/Akt pathway, implies a decreased sensitivity to trastuzumab.[16,17]

EGFR family. Targeted therapies to both ErbB1 (EGFR, HER1) and ErbB2 (HER2) are advanced in clinical trials.[18] In addition, an increasing role of ErbB3 in breast cancer is emerging. ErbB3 is frequently overexpressed in breast cancer and the coexpression with HER2 is considered a poor prognostic indicator. ErbB3 has also been involved in the development of resistance to antiestrogens. It has been also postulated that ErbB3 could be a partner for ErbB2 (HER2) in promoting breast tumour cell proliferation, by a heterodimer formation.[19,20] So far little is known about the role of ErbB4 and its possible clinical relevance.[21] In any case, an evaluation of HER1 and HER3 overexpression will be useful in the future. A strategy for studying prognostic and predictive profiles of breast tumours has been developed using smaller sets of genes. Although results are very interesting, we are probably far to introduce similar technology in diagnostic routine since, so far, the real superiority of this test remains unproven but it is considerably expensive.[22]

Conflict of interest statement

Authors disclose no financial and personal relationship with other people or organisations that could inappropriately influence their work.

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