From conventional pathologic diagnosis to the molecular classification of breast carcinoma: are we ready for the change?

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Abstract
Breast cancer is the most frequent malignant tumor in women and the diagnosis, prognosis and therapeutic strategy are based on the pathologic report. In last years, it was shown that conventional pathologic diagnosis brings few data about prognosis and tells nothing about the response of the tumor to specific therapy. In an effort to improve the molecular characterization of breast cancer, gene profile analysis was performed in a large number of cases. Based on this analysis, there were characterized five molecularly different subclasses: basal-like, luminal type A and B, HER-2, and unclassified. It was shown that prognosis and response to adjuvant therapy is significantly different in these five subtypes. Immunohistochemistry was demonstrated to be a good and acceptable surrogate of the gene analysis. A panel of antibody that includes estrogen receptors (ER), progesterone receptors (PR), Her2 protein, cytokeratin 5 (CK5), epidermal growth factor receptor (EGFR), p53 mutation, and Bcl-2 expression, can discriminate between these five molecular subclasses. In the present review there are presented the main characteristics of the molecular subclasses, the relationships with the conventional pathologic classification, critical problems of the molecular classification and their impact on prognosis and therapy.

Keywords: breast cancer, molecular classification, immunohistochemistry, prognosis, therapy.

Introduction
Breast cancer is nowadays the most frequent malignant tumor in female and morbidity and mortality continue to increase, despite remarkable progresses in the field of early diagnosis and adjuvant therapy. Only in 2006, in US there were reported over 200 000 new cases of invasive breast cancer and more than 40 000 women died of the disease during that same time period [1]. For decades, the conventional pathologic diagnosis was thought to be the “golden standard” in terms of the microscopic subtype and grading. On the other hand, long-term follow-up of patients with breast cancer have shown that a particular subtype of carcinoma or a specific grade has only a minor impact on prognosis and brings almost any information about the therapeutic strategy. Probably the best confirmation comes from the clinic, and we can just note that the conventional pathologic subtype is not included in Nottingham Prognostic Index (NPI). Also, it is very well known that patients with the same pathologic subtype and grade frequently have a different outcome. An explanation might come from histology. More than 25 years ago, many different epithelial cells were characterized in the normal mammary tissue (e.g. basal-like, luminal type A, luminal type B). The term ‘basal’ applies to cells that express basal cytokeratin (CK5/CK14) and luminal cells express only CK8/18, as shown in early studies [2]. CK5+ cells are adult progenitor cells that give rise to intermediary double labeling cells (CK5+/CK8/18+, CK+/smooth muscle actin+) to produce finally fully differentiated cells [3]. This indicates the existence of a progenitor cell in the luminal/over basal compartment, regulated by BRCA1, as hypothesized by Foulkes WD [4]. Each normal cell type can be represented as a phenotype of invasive carcinoma, with a distinct histology and hormone receptor expression.

A significant improvement was achieved in the last decade by investigating some markers useful in pharmaco-diagnosis (ER, PR, Ki67, and HER2). It was found that patients with breast cancer can be stratified based on the expression of these markers, as demonstrated by immunohistochemistry or/and gene analysis [5, 6]. There have been accumulated many evidences that hormone receptors and HER2 expression have direct impact on therapy and no significant correlation was found with conventional subtypes of breast carcinomas [7–9]. It is very likely that this difference be because molecularly distinct diseases are grouped into clinical subtypes, based on their morphology. In an attempt to solve this problem, breast cancers have been classified based on their gene expression profile and immunohistochemical expression...
of cytokeratin (5/6 and 8/18), estrogen receptors, EGFR and HER2. This classification, which recognizes luminal, basal, and HER2 subtypes, had an immediate impact on therapeutic strategies and it was shown that molecular subtypes respond differently to preoperative chemotherapy and to adjuvant postoperative therapy. These data bring new insights into the natural evolution and biology of breast cancer, but the molecular profile of these tumors is far to be complete.

Breast cancer is a heterogeneous disease that includes many morphological and molecular entities. This concept emerged four decades ago from conventional histopathological studies [10], and supported by gene analysis and immunohistochemical findings [11]. The molecular profile of breast cancer should answer to three major questions: (i) do the biology of the tumors differ amongst each other and in comparison with normal tissue? (ii) Can it accurately predict the clinical outcome of patients with morphologic similar tumors? (iii) Can it predict the response to specific therapy in individual cases?

## Molecular subclasses of breast carcinoma

Five distinct molecular subclasses of breast cancer have been identified by gene analysis: estrogen receptor positive that includes luminal A and B tumors, and estrogen receptor negative that includes HER2 type, basal-like tumors and unclassified/normal breast-like tumors [12, 13]. It is not clear yet if the fifth group is really an individual entity, or it includes subtypes not yet characterized [1]. Results obtained with gene analysis overlapped with immunohistochemical findings, based on the expression of hormone receptors (ER and PR), HER2, EGFR, monoclonal cytokeratin, p53 and Bcl-2. Although less sensitive, immunohistochemistry can be a useful surrogate of gene expression analysis. The immunohistochemical panel was validated at the University of British Columbia, in a study performed on 939 patients. Which are criteria for the ‘positive’ reaction using these markers? Many authors agree that tumors considered being positive for ER/PR show nuclear reactivity in over 10% of neoplastic cells, at any nuclear/cytoplasmic ratio, high mitotic index, necrosis and high NPI score [19, 20]. A high proportion of basal-like tumors show metastatic features (90.8%), and also, the majority of cases with medullary carcinoma fall into this type [21–23]. The metaplastic carcinoma shows positive reaction for EGFR, CK5/6, CK14, CK17, and p63 in the majority of cases. By including p63 and CK14 in the immunohistochemical panel, 93.8% metaplastic breast carcinoma can be classified as basal-like tumors. The basal-like carcinoma disseminates less frequently in axillary lymph nodes, liver and bones, and develops metastatic deposits in the brain and lungs [24]. Based on genetic and immunohistochemical analysis, medullary carcinoma seems to be a subtype of basal-like type, based on the triple negative character and CK5/6 expression [25].

A high proportion of triple negative tumors expressed CK18, and could be erroneously considered luminal type B tumors. Preliminary results showed that the triple negative character is not enough to define basal-like carcinoma of the breast. Additionally, basal-like carcinoma expresses CK14, CK17, vimentin (94%) and HER1, but their diagnostic significance was not yet established [26]. Many but not all basal-like tumors stain for both CK5/6 and CK 8/18. It seems that the most common immunphenotype of basal-like carcinoma is ER negative, HER2 negative, vimentin positive, EGFR positive, CK5 positive and CK8/18 positive (Figure 1). From them, the strong vimentin and CK5 expression are the most specific markers for basal-like tumors [19]. In these cases, vimentin expression has also a prognostic value, and correlates with poor prognosis and ER negative status [26]. Specific markers of the myoepithelial cells are not expressed in the large majority of cases.

Despite the fact that basal-like carcinoma is now well characterized, precursor lesions are virtually unknown. DCIS associated to invasive basal-like carcinoma shows the same immunophenotype as the invasive tumor, and this provides evidence for the in situ precursor lesion [6]. Isolated DCIS with basal-like phenotype was found in 6 to 10% of the cases and null phenotype was not yet reported [27, 28]. In these cases, P-cadherin was expressed in 75%, and seems to be a good additional marker for basal-like DCIS. DCIS in this condition is solid, flat or micro papillary with high nuclear grade and necrosis. The absence of atypical ductal hyperplasia and small quantities of DCIS suggests that such a tumor grows very rapidly.

The expression of basal or myoepithelial markers has been reported in 2 to 18% of the cases with ductal invasive carcinoma, and in 25% of G3 breast carcinoma [29, 30]. Some studies showed that basal-like...
carcinoma is associated with a higher risk for brain and lung metastases, higher rate of recurrence and for cancer-related death, independent of lymph node status and tumor size [30–33]. Preliminary results showed that adjuvant anthracyclin-based chemotherapy is less effective for this group and other therapeutic strategies should be considered (identified) for basal-like carcinoma.

Based on the dysfunctional character of BRCA1 and mutation of TP53 gene, it was engineered the conditional mouse BLG-Cre; BRCA1; p53 [34]. BRCA1 gene was inactivated in β-lactoglobulin expressing cells (luminal cells) and in all cells, only one wild type allele of p53 was expressed. Breast tumors developed in this conditional mouse showed a basal-like phenotype in 78% of the cases [35]. Therefore, it was showed for the first time that basal-like carcinoma is an individual and reproducible entity.

**Luminal-like breast carcinoma**

Luminal-like breast carcinoma is characterized by the expression of ER, PR, Bcl-2 and CK8/18. An additional marker, GATA3, is expressed in the luminal type, with higher levels of expression mainly in the luminal A subtype, which shows a more favorable prognosis. This definition of the luminal type carcinoma is independent on the CK5/6 and EGFR, but the expression of these markers may be found in some cases. Less than 9% of cases are of histological grade III [19].

**Luminal type A**

Luminal type A (56–61%) is characterized by high levels of ER expression and is associated to a relatively good prognosis [36]. Many of the genes found in luminal type A breast carcinoma are typically expressed in the luminal epithelium that lines the ducts. The typical immunohistochemical profile of luminal type breast cancer is ER positive and/or PR positive, and HER2 negative. Based on the molecular profile, all cases with pure lobular carcinoma *in situ* are luminal type A tumors [37]. Consecutively, the large majority of invasive lobular carcinomas have a profile characteristic for luminal type A. The immunohistochemical profile of luminal type A breast cancer is shown in Figure 2.

**Luminal type B**

Luminal type B (9–16%) tumors could present a more aggressive phenotype, including high tumor grade [12]. More often, besides ER and/or PR expression, these tumors express EGFR-1, HER2, and cyclin E1 [38, 39].

The molecular classification was further refined based on immunohistochemical data. Almost half of basal-like tumors consist of a mixture of CK5/14 positive and negative tumor cells. Laakso M et al. [39] proposed a new entity, called basoluminal carcinoma that could reflect the differentiation into the luminal phenotype after malignant transformation. Significant differences were noticed between basal-like and basoluminal tumors in terms of cell proliferation (Ki67 index: 33% vs. 58%, respectively), oncogene profile and patient survival.

**HER2 type (8–16%)**

The HER2 positive type includes two distinct subtypes based on the expression of ER: ER negative that cluster near the basal-like tumors (HER2 positive ER negative subtype), and ER positive, which cluster near the luminal B subtype (may express PR). Some cases with HER2 type may also express EGFR [40]. In the majority of the cases, p53 is not expressed by tumor cells, and the expression of CK8/18 is heterogeneous and moderate. If positive, reaction for EGFR is focal and restricted to a minority of less than 5% of tumor cell population (Figure 3).

HER2 type is frequently associated with DCIS, many cases are less differentiated and are characterized by poor prognosis [41].
Normal breast-like type/unclassified (6–10%)

In the normal breast, basal cells stain with cytokeratin 5/6 and luminal cells stain with cytokeratin 8/18 [37]. Basal cells probably represent a mixture of different cell types with high proliferative potential and luminal cells are more differentiated. Whether these cell types include a stem cell population capable of self-renewal is still unknown. Normal breast-like carcinoma is a triple negative tumor and is close to basal-like carcinoma in terms of the molecular profile, a slightly better prognosis than basal-like type, and does not respond to neoadjuvant therapy. It is important to point out that the term ‘unclassified’ used in this classification has nothing to do with ‘not otherwise specified’. The unclassified type is negative for all five markers: ER, PR, HER2, CK5 and EGFR. In terms of the immunohistochemical profile, outcome and survival, these tumors are close to the basal-like carcinoma.

The relationships between the molecular subtypes and ER, PR, HER2 status, nuclear grade and mitotic index are shown in the Table 1. As it can be noticed from the table, there is a considerable overlap between basal-like and unclassified carcinoma, except for the nuclear grade and mitotic index, both significantly higher in the basal-like type. On one hand, these data show the existence of the so-called ‘triple negative’ tumors (ER, PR and HER2 negative), and on the other hand, it shows that not all triple negative tumors are basal-like.

Triple negative phenotype

Triple negative phenotype includes all breast cancers that lack ER, PR and HER2 expression and represent 10 to 17% of all breast carcinomas, and 96.8% of them are of histological grade 3 [42]. The prevalence of triple negative tumors is 23% in patients under the age of 40, 16% for patients aged 40–49, and 11% for patients over 50 years.
For therapy.

Recently, dasatinib, a src inhibitor, has been found effective in breast cancer cell lines with triple-negative phenotype. This subtype seems to originate in apocrine carcinoma in six (14.28%), HER2 type in seven (16.66%), and unclassified in five (11.9%) cases. Additionally, we performed CK8/18, Bcl-2, and p53 in molecular types of breast cancer (Rakha EA et al., 2008 [58], modified)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Basal-like (n = 22)</th>
<th>HER2+/ER- (n = 7)</th>
<th>Luminal A (n = 22)</th>
<th>Luminal B (n = 2)</th>
<th>Unclassified (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER status</td>
<td>0</td>
<td>4</td>
<td>86%</td>
<td>97%</td>
<td>0</td>
</tr>
<tr>
<td>PR status</td>
<td>100%</td>
<td>100%</td>
<td>14%</td>
<td>3%</td>
<td>100%</td>
</tr>
<tr>
<td>HER2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Nuclear grade ++</td>
<td>80%</td>
<td>76%</td>
<td>26%</td>
<td>27%</td>
<td>65%</td>
</tr>
<tr>
<td>Nuclear grade +</td>
<td>20%</td>
<td>24%</td>
<td>74%</td>
<td>73%</td>
<td>35%</td>
</tr>
<tr>
<td>Mitotic index &gt;10</td>
<td>87%</td>
<td>69%</td>
<td>31%</td>
<td>32%</td>
<td>52%</td>
</tr>
</tbody>
</table>

The evaluation of the molecular profile on large series has demonstrated that 'triple negative' tumors fall into the basal-like and unclassified tumors. A subset of 'triple negative' tumors do not express CK 5/6 or EGFR, and therefore, the 'triple negative' phenotype is not enough to characterize a basal-like carcinoma [43]. From the practical point of view, it is important to remember that triple negative is not equivalent to basal-like carcinoma. The diagnosis of these tumors has the advantage that these three stains are already routinely used to guide the therapeutic strategy. Recent data have shown that lymph node status is a less reliable predictor of prognosis in 'triple negative tumors', and cytokeratin 5/6 and EGFR expression may provide a better marker for long term prognosis [32]. The aggressive character of this type of tumor is demonstrated by the pick of recurrences that occur between 1 and 3 years, and the majority of deaths occur in the first 5 years, following therapy. The unfavorable prognosis is also supported by the fact that the majority of triple negative cases are predominantly of histological grade 3 [44]. It becomes evident that triple-negative tumors form a heterogeneous group, and 56 to 84% from them express CK5/6 and EGFR. The only systemic therapy currently available for patients with such tumors is chemotherapy. The unfavorable prognosis is also supported by the fact that the majority of triple negative cases are predominantly of histological grade 3 [44].

In a pilot study we performed immunohistochemical evaluation of 42 cases with invasive breast carcinoma, testing the reactivity for ER, PR, HER2, CK5/6, EGFR, p53 and Bcl-2, which are compulsory to obtain a molecular classification. We found luminal A type in 22 (52.38%) cases, luminal B in two (4.76%), basal-like carcinoma in six (14.28%), HER2 type in seven (16.66%), and unclassified in five (11.9%) cases. HER2 type associated with ER positive was found in two cases, and associated with ER negative in four cases. Additionally, we performed CK8/18, Bcl-2, and p53, to demonstrate their possible significance in classification of molecular types of breast cancer. Results are shown in Table 3.

<table>
<thead>
<tr>
<th>Class</th>
<th>Incidence</th>
<th>Important aspects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminal A</td>
<td>56–61%</td>
<td>Highest ER expression, best prognosis</td>
</tr>
<tr>
<td>Luminal B</td>
<td>9–16%</td>
<td>Low/moderate ER expression, Ki67 ++, B+/A, genes shared with the basal-like and HER2, less favorable outcome</td>
</tr>
<tr>
<td>Basal-like</td>
<td>8–20%</td>
<td>Expression of CK5, CK17, c-kit, EGFR, P-cadherin, p53 mutations, BRCA1 mutations</td>
</tr>
<tr>
<td>HER2+</td>
<td>8–16%</td>
<td>High levels of p53 mutation, aggressive behavior, poor prognosis, do not respond to hormonal therapy</td>
</tr>
<tr>
<td>Normal breast-like/unclassified</td>
<td>6–10%</td>
<td>High expression of genes characteristic of parenchymal basal epithelial cells and mesenchymal cells; low expression of genes characteristic of luminal cells; prognosis better than of basal-like tumors; respond to neoadjuvant chemotherapy at lower rates than other ER negative tumors</td>
</tr>
</tbody>
</table>

An additional molecular subtype of breast cancer could be the so-called apocrine carcinoma, characterized based on androgen receptor (AR) expression. This subtype seems to originate in apocrine glands of the axilla and three subtypes were identified: ER+ AR+, ER- AR-, and ER- AR+. In the molecular classification, this subtype replaces HER2+ ER- type, previously described [45, 46]. The androgen receptor-based classification requires further investigation but suggests another molecule that may be a future target for therapy.
The immunohistochemical expression of CK5/6 is not restricted to basal-like carcinoma, and it was found in all types, except for the unclassified tumors. Somehow, surprisingly, CK5/6 was positive in 33% of luminal type carcinoma, and this supports the existence of the basoluminal type, previously reported by others [39]. It must be noticed that more than a half of our HER2 cases expressed CK5/6 and Bcl-2 (the last is usually found in the luminal type). CK8/18 is expressed in all types, and therefore it seems to be less useful for this classification. EGFR expression was found in one case with basal-like carcinoma and one classified as luminal B. Although there were few cases included in this preliminary study, the expression of EGFR in luminal B type may suggest the existence of the basoluminal subtype.

Practical protocol for the diagnosis of breast cancer subtype

The diagnosis of the molecular subtype of breast cancer is based on the expression of ER/PR, HER2, cytokeratin 5 and EGFR, as shown in the table below (Table 4).

Table 4 – Practical protocol for the molecular diagnosis of breast cancer (after Conforti R et al., 2007 [47], modified)

<table>
<thead>
<tr>
<th>ER+ breast cancer (luminal type)</th>
<th>Should be followed by splitting luminal A/B</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER+ HER2+</td>
<td>HER2+/ER- breast cancer (HER2 type)</td>
</tr>
<tr>
<td>ER- HER2-</td>
<td>HER2-/ER-</td>
</tr>
<tr>
<td>CK5+/EGFR+ (basal-like)</td>
<td>HER2-/ER-</td>
</tr>
<tr>
<td>CK5-/EGFR- (non-basal breast cancer)</td>
<td></td>
</tr>
</tbody>
</table>

Additional and useful information may be provided by the expression of cytokeratin 18, Bcl-2 and p53. Using this relatively simple system of classification, in a study on 935 patients, Conforti R et al. [47] found the luminal-like type (ER+) in 66% of cases, basal-like in 12%, HER-/ER- non-basal in 14%, and HER2 subtype in 8%. The luminal-like and HER2-/ER- subtypes were not further refined by the expression of additional markers, but on the other hand, this protocol may be applied to a large number of cases. Perhaps the most important result of this finding is related to the accuracy of the combination of these four markers in order to define basal-like carcinoma. Another practical aspect is related to the characterization of the HER2-/ER- non-basal subtype, an aggressive tumor found mainly in younger patients that also exhibit some characteristics of the luminal type (CK18 expression).

Molecular classification, prognosis and survival

The prognosis of patients with breast cancer based on the molecular classification is still a problem of debate. Some points have clinical relevance, and here, it must be mentioned that ER+ tumors include a subtype (luminal B) that show a poorer prognosis than luminal type A carcinoma. Triple-negative tumors are to be classified as basal-like, normal breast-like/unclassified, each with a distinct molecular profile and outcome. ER status is strongly associated with gene expression, and tumor grade shows a moderate association. There is no strong evidence that nodal and menopausal status of the patient or tumor size is associated with the expression profile of the tumors [48]. A significant better prognosis was found in patients with luminal carcinoma and significant differences were found between the two subtypes with ER positive tumors [36, 49].

Long-term follow-up studies were performed only retrospectively, due to the novelty of the concept. In a study on 496 cases, it was found the following data regarding breast cancer specific survival (8.1–11.2 years follow-up): basal-like 75%, HER2+/ER- 52%, luminal A 84%, luminal B 87%, and unclassified 77% [14]. Like for the ER, PR and HER2 status, a non-significant difference was found between basal-like and unclassified carcinoma. Differences in survival were more evident if analysis was based on the lymph node status (Table 5).

Relation between molecular classification and adjuvant treatment

For years, an important question in the clinical management of breast carcinoma was how to avoid overtreatment and undertreatment, based on the observation that treatment failure occurs in approximately 30% of patients [50]. ER positive cases are treated with hormone therapy, but on the other hand, luminal type responds poorly to chemotherapy. The response of ER negative patients to chemotherapy is not uniform, and this suggests that both ER positive and negative breast cancers need to be subdivided by tumor biology and response to therapy [43, 51]. In the HER2 positive group, the treatment with trastuzumab significantly improved the prognosis and combined with chemotherapy, it was noticed a remarkable reduction in relapse. Not all HER2-positive cases respond to trastuzumab therapy and resistance maybe induced by PTEN loss or CXCR4 up regulation. Anthracycline-based chemotherapy associated or not with ER inhibitors has become a standard treatment, but a large proportion of patients do not benefit from this therapy. It stands out the necessity to identify new predictive biomarkers. Data about the efficacy of preoperative chemotherapy related to the molecular classification are still controversial. In some studies, it was reported that the molecular subtype predicts the response to preoperative chemotherapy [52] and in others, only ER status was found to be useful [47]. In a large study, it was shown that basal-like and HER2 tumors are more sensitive to neoadjuvant anthracycline-based chemotherapy than luminal types [14]. On the other hand, it was demonstrated that up to 45% of basal-

### Table 5 – The relation between the lymph node status, molecular subtypes and survival

<table>
<thead>
<tr>
<th>Lymph node status</th>
<th>Basal-like</th>
<th>HER2+/ER-</th>
<th>Luminal-like</th>
<th>Unclassified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>51%</td>
<td>39%</td>
<td>65%</td>
<td>83%</td>
</tr>
<tr>
<td>Negative</td>
<td>93%</td>
<td>71%</td>
<td>94%</td>
<td>92%</td>
</tr>
</tbody>
</table>
like tumors show complete response after 12 weeks of paclitaxel followed by neoadjuvant chemotherapy with 5-Fluorouracil, doxorubicin and cyclophosphamide [52]. In this study, basal-like and HER2 types were associated with the highest rate of pathological complete response (45% for both), and luminal type showed a lower rate of complete response (6%). In the normal breast-like type, no complete response was noticed. The molecular profile performed before surgery on samples taken by fine needle biopsy was demonstrated to be useful in the prediction of response to chemotherapy. Using this method, Sotiriou C et al. [53] were able to discriminate between responders and non-responders to Adriamycin and cyclophosphamide-based chemotherapy. This method can also be easily applied in order to monitor post-chemotherapy changes as a measure of the pharma-dynamic effect. Unfortunately, none of the biomarkers is strongly predictive of chemotherapy response in cases with metastatic disease, but survival seems to be dependent on the hormone receptor and p53 status [54, 55].

Targeted therapy became largely applied in the last decade. EGFR tyrosine kinase inhibitors might be a viable treatment option and clinical trials were initiated, based on gefitinib and erlotinib. A subset of basal-like carcinoma expresses c-kit, is associated with better prognosis, and therefore, targeted therapy could be initiated [18, 56]. Until now, only disappointing breast cancer response rates were reported. C-kit positive tumors usually fall into the category of basal-like carcinoma (the so-called adenoid cystic carcinoma), and patients were treated with Imatinib or Sunitinib. The efficacy of targeting c-kit will probably depend on the prevalence in the tumors or its role as predictive marker of response (both aspects are largely unknown).

Recently, it was shown that anti-vascular endothelial growth factor, bevacizumab, improves survival in metastatic breast cancer when combined with paclitaxel [57]. More than 60% in this study were hormone receptor positive and none were HER-positive, suggesting that antiangiogenic strategies may be effective in the luminal type tumors. It becomes clear that there is a need to identify new specific therapeutic targets. Only by using the best of the old as well as the best of the new will ensure the maximal therapeutic benefit with the least possible risk of adverse side effects.

Future directions and perspectives

How novel is this molecular classification? What is the clinical significance of the classes identified? How much additional information does the classification offer over traditional methods, and is this important for the patient management? Does it really outperform current classification systems? These and other questions of academic and clinical interest are yet to be satisfactorily answered [58]. The new molecular classification brought new insights into the biology and behavior of breast cancer. At present time, there are characterized at least five types of mammary carcinoma, based on gene analysis and immunohistochemical profile. Despite the fact that this classification correlates with prognosis, there are still many questions to be answered. Maybe the first of them is related to the possibility to replace the conventional morphologic classification. At this moment, the answer is probably no, because some types are not fully characterized and it is very possible that some of them (e.g. basal-like and unclassified) include different subtypes. Moreover, such a change may create confusion, mainly because some types are not completely characterized. A good example is represented by the expression of EGFR and c-kit that could represent viable targets for therapy, both found in some but not all cases with basal-like carcinoma. Results on their immunohistochemical expression are still controversial. As preliminary results in clinical trials with EGFR inhibitors were disappointing, there is a need to identify new molecular targets. Another point is related to the molecular profile of unclassified tumor, in which all five basic markers are negative, and therefore, therapy is restricted to adjuvant chemotherapy and/or radiotherapy. It is still not clear the distinction between ‘unclassified’ and ‘normal breast-like’ tumors, included by some authors in the same type or the last that is not mentioned by others. If the “normal breast-like” cancer does really exist, which are its molecular profile and the relation with luminal type cancer? It is an unanswered question until now. Another question: what should be done with some well-known pathologic forms of breast carcinoma, like papillary, medullary, or mucinous? Probably this is the easiest answer: nothing, because they fall in one of the five types, based on their molecular profile.

Is there any take home message? Maybe the following: luminal and HER2 types are well characterized and they strongly correlate with prognosis and response to specific therapy. Basal-like carcinoma is a distinct entity that has a significantly higher rate recurrence, a shorter disease-free and overall survival, and chemotherapy seems to be less effective in this group. Further studies are necessary to clarify the molecular profile of basal-like, and mainly of unclassified breast cancers.

References


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