Molecular Classification of Breast Cancer

Lars A. Akslen

Centre for Cancer Biomarkers CCBIO
Department of Clinical Medicine, Section for Pathology
University of Bergen - Haukeland University Hospital
Email: lars.akslen@k1.uib.no

The primary goal of breast cancer classification is to provide information that can guide the treatment of patients and be useful in prognostication. Traditionally, there is a wide variation in the morphology of breast cancer, and some subtypes and variants show typical clinico-pathologic correlations. Recent information on the molecular characteristics and phenotypes of human breast cancer has emerged, and some of this information is relevant for patient management in the era of precision medicine.

In 2000, Perou and co-workers published a key paper proposing that breast cancers might be classified into certain "intrinsic" subtypes based on gene expression patterns. The following subgroups were identified: ***1. luminal-A subgroup; 2. luminal-B subgroup; 3. HER2 subgroup; 4. basal-like subgroup; 5. normal breast-like subgroup.*** In later studies by different groups, these categories have been largely confirmed and validated. Especially, the HER2 subgroup and the basal-like (BL) subgroup have been focused due to their poor prognosis and lack of targeted treatment for basal-like cancers. Subsequently, surrogate markers by immunohistochemistry were shown to correlate well with the original "intrinsic" subtypes. The St Gallen 2013 consensus guidelines present the molecular classification based on IHC markers (ER, PR, HER2, Ki67). Further, genomic studies have suggested as many as 10 subtypes of breast cancer.

The ***luminal subgroups*** (~70%) are typically, but not always, characterised by positivity for estrogen (ER) and progesterone (PR) receptors, and the prognosis is often favourable, especially for the luminal-A category. The luminal-B category is ER positive but can be negative for PR, show HER2 positivity, or have high Ki67 (20% or above) and has a poorer prognosis. How to apply Ki67 in this subclassification is controversial.

The ***Her2 subgroup*** (~15-20%) is identified by strong membrane expression of the HER2 protein (score 3+) or gene amplification by in situ hybridization techniques. These alterations predict for response to anti-HER2 treatment, and this is a good example of *targeted diagnostics and therapy* and the importance of routine pathology for providing predictive markers as a guideline to stratify patients for treatment. The HER2 subgroup is shown to be heterogeneous but no subclassification is presently used.

The ***basal-like subgroup*** (~10-15%) has been examined in many studies and is also known to be heterogeneous *1. Criteria and definitions.* Originally, this category was defined by mRNA expression of markers found in basal cells or myoepithelial cells in

the normal breast. In the first validation study (van de Rijn, 2002), basal differentiation by immunohistochemistry was defined as expression of cytokeratin (CK) 5/6 or CK17, and this subgroup showed a significantly poorer prognosis. Others have used single markers like CK5/6 or P-cadherin (Arnes, 2005). The Nottingham group used the combination of CK5/6 and CK14 to define BL tumors (Rakha et al., 2006). Others use a marker profile to define the BL category, like the *"core basal profile"* as defined by Nielsen et al. in 2004: ER negative, HER2 negative and CK5/6 and /or EGFR positivity. Also, the *"triple negative profile"* has been increasingly used (ER neg., PR neg., HER2 neg.), and a substantial proportion of these cases (~80%) show positive expression of basal markers. *2. Morphology.* Basal-like breast cancers appear to be associated with high-grade histologic features. Due to their association with *BRCA1* mutations, features such as pushing borders, high-grade cytology and tumor infiltrating lymphocytes are increased. Finally, it is known that breast cancer subtypes such as adenoid cystic carcinoma and metaplastic carcinoma often have a basal-like differentiation. *3. Association with BRCA1 positive breast cancer.* Foulkes et al. (2003) showed that the expression of CK5/6 protein, as a marker of basal differentiation, was significantly associated with *BRCA1* germline mutations. This might be of practical importance in selecting patients for *BRCA1* tests. Lakhani et al. later confirmed these findings and showed that a combination of ER, CK5/6 and CK14 might strongly improve the prediction of *BRCA1* status (2006). *4. Metastatic pattern.* Studies have indicated that the frequency of lymph node metastases might be decreased and hematogenic metastases increased among basal-like breast cancers. In our previous study (Foulkes et al., 2003), lymph node metastases was less frequent in the basal-like subgroups as defined by CK5/6 expression (OR=0.27). This might explain the shape of the survival curve for BL tumors, rapidly falling and then levelling off, as shown in several studies (e.g. Rakha et al., 2006). *5. Angiogenic phenotype.* Studies have indicated that the basal-like subtype might be associated with markers of increased angiogenesis like vascular proliferation and a glomeruloid microvascular phenotype. This vascular pattern is also associated with the *BRCA1* positive genotype. *6. Other molecular alterations.* Several molecular markers appear to be associated with BL tumors. Nielsen et al. (2004) showed that the expression of EGFR and c-KIT was increased in basal-like cancers. Also, markers such as p63, SMA, Caveolin-1 and Cyclin E are increased in this subgroup. *6. Prognosis.* Studies have shown that the prognosis is significantly decreased among basal-like breast cancers (e.g. Rakha, 2006), even in multivariate analysis when compared with standard variables such as tumor size, histologic grade and lymph node status.

Studies so far indicate that breast cancers with a basal-like differentiation should be considered as a heterogeneous group, in addition to the fact that there is still no consensus on how to define the basal-like subgroup. St. Gallen 2013 suggests that the *triple negative category* might be used as a surrogate definition for the basal-like category due to ~80% overlap, but it is not known whether this is sufficiently precise. Burstein el al. (2015) recently described 4 subgroups of triple-negative cancers: luminal androgen receptor subtype, mesenchymal subtype, basal-like immuno-suppressed subtype, and basal-like immunostimulated subtype. Claudin-low and molecular apocrine categories have also been mentioned. Other studies indicate that the mesenchymal subtype show some stem-cell characteristics.

Pathology of hereditary breast cancer. Studies have shown that breast cancers with germline mutations of the *BRCA1* gene appear to have a specific morphologic

phenotype, characterized by increased frequency of classical medullary carcinomas and individual medullary features such as pushing borders, higher cytologic grade, and lymphocytic infiltration. As mentioned above, *BRCA1* positive breast cancers are significantly associated with a basal-like phenotype. These features might assist in selecting patients for genetic tests.

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