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19 Genomic Markers ER-Negative Breast Cancer

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Abstract

In this chapter we will cover the role and value of genomic markers in the ER-negative subset of breast cancer. Such genomic markers encompass several different types of molecular alterations. The markers may represent proteins that can be detected by immunohistochemistry, as for example the progesterone receptor (PR), the androgen receptor (AR), or HER2. Other types of genomic markers included in this overview are markers based on gene expression data obtained from profiling breast tumor mRNA or small RNAs, as well as respective genomic tests based on such expression profiles. Furthermore, mutations in cancer genes, either hereditary or somatic, will also be covered in this chapter because of their potential prognostic and predictive value. Those mutations may represent single altered genes or mutational patterns or structural variations that have been identified through recent whole genome sequencing efforts. Regarding the value of genomic markers in ER-negative breast cancer we distinguish between risk factors for cancer susceptibility on the one hand, and factors with prognostic or predictive value on the other. Finally, we discuss the important but complex role that immune infiltration may have in ER-negative breast cancer. What we do not cover however are standard clinicopathologic factors, such as histopathological grading or age, which undoubtedly also have an important prognostic role in addition to the genomic markers discussed here.

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Keywords

Breast cancer · ER negative · Triple negative subtype · HER2 subtype · Genomic markers · Prognosis · Gene expression signatures · Mutation signatures · Prognosis · Tumor infiltrating lymphocytes

19.1 Breast Cancer Subtypes

Breast cancer is a heterogeneous disease consisting of different molecular subtypes, each having a distinct natural history and clinical behavior. These subtypes are recognized based on histological characteristics as well as on molecular markers (Weigelt and Reis-Filho 2009). Currently the simplest and clinically most useful stratification of breast cancer is based on expression of the hormone receptors for both estrogen (ER) and progesterone (PgR) as well as the human epidermal growth factor receptor 2 (HER2) determined by immunohistochemistry (IHC) methods (Sotiriou and Pusztai 2009). Based on these three receptors tumors are characterized as hormone receptor–positive, HER2-positive (i.e., amplification or overexpression of HER2), or triple-negative breast cancer (TNBC) lacking the expression of all three receptors. In addition several refined stratifications applying genomic methods or the inclusion of additional immunohistochemical markers (e.g., Ki67) allow the distinction of "Basal-like" breast cancers as well as "Luminal A" and "Luminal B" subgroups each with different prognosis and clinical behaviour (Perou et al. 2000; van't Veer et al. 2002; Prat et al. 2012; Reis-Filho and Pusztai 2011; Kaufmann et al. 2011). The basal-like and HER2-like subtypes are highly proliferative and have a poor prognosis if untreated, but exhibit an increased sensitivity to chemotherapy (Perou et al. 2000; Sorlie et al. 2001; Rouzier et al. 2005; Rody et al. 2007). Still the additional clinical value of molecular classification is limited by its close correspondence with the status of ER, PR, and HER2, along with tumor grade (Sotiriou and Pusztai 2009). Relatively high concordance (75–90 %) exists between molecular subtypes as defined by genomic methods and IHC phenotype (Reis-Filho and Pusztai 2011). Following either of these subtyping methods, the main two classes of ER-negative breast cancers are triple-negative or basal-like cancers on one hand, and HER2-positive cancers on the other. These two subtypes are fundamentally different in their biology and current clinical management and thus should be considered separately. This is of major importance given the lack of targeted therapies for TNBC and the various HER2-targeted therapeutic approaches. Consequently, HER2 amplification represents the most important genomic marker in ER-negative breast cancer to distinguish HER2-positive from triple-negative disease.

19.2 Hormone Receptor Subtypes Within ER-Negative BC

Expression of the steroid hormone receptors (HR) has long been recognized as important in the clinical management of breast cancer, having both prognostic and predictive implications for endocrine therapy. The American Society of Clinical Oncology and the College of American Pathologists recommend testing for both estrogen receptor (ER) and progesterone receptor (PR) on all newly diagnosed invasive breast cancer cases (Hammond et al. 2010). Although the importance of ER expression is well established, the clinical significance of PR expression remains controversial, especially in ER-negative breast cancer. PR expression has been hypothesized to be associated with good prognosis in certain types of HR-negative invasive carcinoma, such as adenoid cystic carcinoma and secretory carcinoma, which generally have excellent prognosis (Rakha et al. 2007b). Compared to ER−/PR−

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tumors, ER−/PR+ tumors appear to have a more favorable prognosis, lower proliferation and absence of vascular invasion but no significant difference in overall survival (Rakha et al. 2007b). In a large meta-analysis of 21,457 women with early stage breast cancer from 20 randomized trials with adjuvant tamoxifen, PR expression was not predictive of benefit from tamoxifen treatment in ER-negative breast cancer, although there was a slight early benefit from tamoxifen in ER−/PR+ but it was not statistically significant (Early Breast Cancer Trialists' Collaborative et al. 2011).

The conflicting results have raised the possibility that the ER−/PR+ classification is primarily a technical artifact caused by false-negative ER results (De Maeyer et al. 2008). In fact, with the more recent definitions of ER-positivity as minimal (1 %) ER expression, the proportion of cases reported as ER−/PR+ have decreased from about 4 % in the early 1990s to only 1 % in the recent SEER cancer registry data (Early Breast Cancer Trialists' Collaborative et al. 2011). A recent study that integrated gene expression and clinicopathologic data from 20 studies reported that PR is among the least variably expressed genes in ER-negative breast cancer and that ER−/PR+ is by far the least reproducible subtype by a secondary method (Hefti et al. 2013). Therefore, given the rarity and the questionable biological significance of the ER−/PR + phenotype, the clinical use of PR expression in ER− breast cancer is uncertain (Olivotto et al. 2004).

In addition to ER and PR, another nuclear steroid hormone receptor, the androgen receptor (AR), is widely expressed in 70–90 % of all breast cancers (Brys 2000). The role of AR as a prognostic factor or as a potential therapeutic target in breast cancer is controversial and depends on the ER status (Fioretti et al. 2014; Shah et al. 2013). In ER/PR-positive tumors expressing AR, activation of AR with the androgen dihydrotestosterone appears to decrease estrogen-dependent signaling, likely through translocation to the nucleus and competition with ER and PR for binding to the estrogen-related elements, thus reducing cell survival and promoting apoptosis. In ER-negative breast cancer, expression of AR varies widely from 9 to 50 %, and about 10–40 % of TNBC express AR (Shah et al. 2013). The effect of AR expression remains rather controversial. Molecular profiling had identified a subgroup of ER-negative/AR-positive breast tumors that had histological apocrine features and was termed the molecular apocrine subtype (Farmer et al. 2005b). This subgroup demonstrated a molecular profile consistent with increased androgen signaling and which resembled that of ER-positive tumors. Based on this, it was hypothesized that signaling though AR replaces, or at least mimics, ER-signaling and transcriptional activation through involvement of the transcription factor FOXA1 (Robinson et al. 2011) promoting cell growth. Furthermore, AR expression appears to be particularly enriched in ER-negative/HER2-positive tumors (Niemeier et al. 2010). In ER-negative/HER2-positive tumors expressing AR, androgens and AR can stimulate oncogenic Wnt and HER2 signaling pathways by FOXA1-dependent transcriptional upregulation of WNT7B and HER3 (Ni et al. 2011). These studies provided justification for targeting AR as a therapeutic strategy in patients with ER-negative or ER-negative/HER2-positive disease. A recent single-arm phase II study that evaluated the effect of the antiandrogen bicalutamide in ER-negative/PR-negative metastatic breast cancers expressing AR reported a 6-month clinical benefit rate of 19 % (Gucalp et al. 2013). TNBC tumors expressing AR also appear to be associated with a significantly higher frequency of activating PIK3CA mutations (40 vs. 4 % in AR-negative) and concurrent amplification of the PIK3CA locus, suggesting the use of AR antagonists in combination with PI3K/mTOR inhibitors as a potentially effective treatment strategy (Lehmann et al. 2014). However these strategies have yet to be tested in the clinic.

Several studies have investigated the prognostic and predictive value of AR expression in ER-negative breast cancer, but the results appear conflicting (Shah et al. 2013; Vera-Badillo et al. 2014). In an ER-negative cohort of 303 post-menopausal women derived from the Nurses'

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Health Study, 43 % of these tumors were AR-positive, but no significant association was found between AR expression and breast cancer specific mortality (Hu et al. 2011). In another cohort of 287 patients with resectable TNBC, 26 % of the cases were AR-positive and these patients had disease free survival that was significantly longer than that of patients with AR-negative breast cancer (He et al. 2012). Another single-institution study involving 282 TNBC tumors, AR expression was demonstrated in 13 % of the cases. Absence of AR expression was significantly associated with higher histologic grade, recurrence and development of distant metastases (Rakha et al. 2007a). A meta-analysis of 19 studies involving 7693 women with breast cancer reported expression of AR in 32 % of the ER-negative cases. Among ER-negative cases, there was a trend towards better 5-year overall (OS) and disease free survival (DFS) with AR expression, but the association did not reach statistical significance in either case (Vera-Badillo et al. 2014). In terms of predictive effects, results from the GeparTrio trial of early stage breast cancer women treated with neoadjuvant docetaxel/doxorubicin/cyclophosphamide, showed that among TNBC patients who achieved complete pathologic response (pCR), those with AR-positive tumors had a DFS of 100 % compared to 79 % of AR-negative tumors (Loibl et al. 2011). However, AR status was not a significant predictor of pCR rate in TNBC, as AR-positive TNBC tumors had a pCR rate of 29 % compared to 33 % in AR-negative TNBC tumors (Loibl et al. 2011). Overall, an emerging volume of evidence suggests that AR plays an important role in carcinogenesis and, as such, it could be a significant prognostic factor and may be further exploited as a novel therapeutic target in ER− disease. However, the plethora of controversial results suggests that further standardization in the estimation of AR expression, scoring systems and cut-off values would be required (Anestis et al. 2015).

19.3 Gene Expression Based Genomic Markers in Different Breast Cancer Subtypes

The clinical utility of currently available genomic tests in ER-negative breast cancer is limited since their main value is in the prognostic stratification of luminal ER-positive tumors (Prat et al. 2012; Cobain and Hayes 2015). For example, the Amsterdam 70-gene signature (Mammaprint) and the Oncotype recurrence score classify almost all ER-negative cancers as high risk. Similarly, the Genomic Grade Index, Breast Cancer Index, and EndoPredict assays are useful only in ER-positive patients (Prat et al. 2012; Gyorffy et al. 2015). While most available multigene prognostic gene signatures may provide standardized, complementary information to routine pathological variables that could assist therapeutic decision-making in ER-positive cancers, they have only very limited utility in ER-negative disease. One reason may be that these so called "first generation signatures" were developed in mixed cohorts including different subtypes, the majority of which being ER positive (Sotiriou and Pusztai 2009). It became increasingly clear that the subtype composition of a dataset can strongly influence the prognostic and predictive gene signatures derived from it (Weigelt et al. 2012). Often these "first generation" signatures represent a surrogate marker for the subtype distinction itself (Prat et al. 2012; Reis-Filho and Pusztai 2011). As a consequence subsequent guidelines have suggested to analyze subtypes of breast cancers separately and to derive subtype-specific genomic tests (Kaufmann et al. 2011; Goldhirsch et al. 2011). However, it has even been suggested that information on some problems may be lacking from the gene expression space (Hess et al. 2011), particularly for ER breast cancer that appears to be transcriptionally more heterogeneous than other subtypes (Jiang et al. 2014; Tofigh et al. 2014).

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19.4 Gene Expression Signatures Developed in ER-Negative Breast Cancer

The realization that the different subtypes of breast cancer are fundamentally distinct in their transcriptional profiles led several groups to investigate these subgroups separately, leading to so-called second generation signatures (Reis-Filho and Pusztai 2011; Alexe et al. 2007; Teschendorff et al. 2007; Finak et al. 2008; Desmedt et al. 2008; Bianchini et al. 2010a; Lehmann et al. 2011; Hatzis et al. 2011; Rody et al. 2011; Karn et al. 2011). Some second generation prognostic signatures for TNBC could identify a subset of cases that had good prognosis when treated with standard of care chemotherapy, but since 20–25 % of these cases were predicted to relapse within 5 years the clinical utility of these signatures was rather limited (Hatzis et al. 2011). Many of these studies identified immune cell infiltration as an important component for prognosis and prediction in ER-negative subtypes. In triple-negative breast cancer studies also identified several subgroups besides immune cell components that can be clearly separated based on transcriptional profiles. Triple-negative disease seems to be composed of basal-like cancers, a molecular apocrine group, and the claudin-low subtype (Farmer et al. 2005b; Lehmann et al. 2011; Rody et al. 2011; Prat et al. 2010; Burstein et al. 2015). Potential therapeutic relevance of these subgroups has been suggested (Vidula and Rugo 2015; Ng et al. 2015). In contrast to these relatively stable separable groups, immune cell infiltration seems to represent a rather continuous parameter and may be detected within all three of these subgroups (Rody et al. 2011; Denkert et al. 2010; Karn et al. 2015). For ER-negative/HER2-positive disease an important role of immune cells has also been demonstrated (Alexe et al. 2007; Ignatiadis et al. 2012; Loi et al. 2014; Denkert et al. 2015). Yet, despite refinements in the definition of ER-negative subtypes, the efforts to define clinically useful prognostic signatures in ER-negative breast cancer has had limited success (Pusztai et al. 2015).

19.5 The Role of Immune Cell Infiltration as a Marker in ER-Negative Breast Cancer

Until recently, molecular and clinical subtyping of breast cancer was solely based on the molecular features of the cancer cells without considering the importance of stromal components, such as tumor infiltrating immune cells (Perou et al. 2000; Kaufmann et al. 2011). However, an association between cancer and immune response components has long been observed (Balkwill and Mantovani 2001). Different immune cells may have either anti-tumor or tumor-promoting effects (Grivennikov et al. 2010). It is also important to recognize that the role of tumor infiltrating lymphocytes (TILs) can differ by breast cancer subtype (Karn et al. 2011; Cancer Genome Atlas Network 2012). Gene expression signal originating from immune cells is easily recognized in high throughput transcriptional profiling data, and the first microarray analyses of breast cancer tissues had already described signatures of TILs (Perou et al. 1999, 2000; Hu et al. 2006). Later on, several larger microarray studies with clinical follow up and meta-analyses revealed the strong positive prognostic value of immune signatures in ER-negative tumors (Desmedt et al. 2008; Lehmann et al. 2011; Rody et al. 2009, 2011; Schmidt et al. 2008; Bianchini et al. 2010b; Nagalla et al. 2013). The prognostic significance of immune signatures was subsequently validated with direct histological and immunohistochemical assessment of TILs and other immune components and are also in line with several earlier studies (Loi et al. 2013, 2014; Adams et al. 2014; Aaltomaa et al. 1992; Menard et al. 1997). The common theme that emerges from all these studies is a significant association of an increasing number of TILs at the tumor stroma with improved patient prognosis. It should be noted that both the presence of immune cell infiltration and its prognostic value are characteristics mainly of ER-negative cancers (Karn et al. 2015). Moreover, increased presence of TILs has been found to be predictive of improved response to neoadjuvant chemotherapy, again mainly in ER-negative tumors

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(Denkert et al. 2010; Issa-Nummer et al. 2013). Finally, for HER2-positive disease, there appears to be an association of lymphocyte infiltration with benefit from trastuzumab (Loi et al. 2013; Perez et al. 2015). Thus, the "prognostic" value of TILs in ER-negative breast cancer may result from "pure prognostic" or "pure predictive" effects or a combination of both.

19.6 Complexity of Immune Cell Markers in ER-Negative Breast Cancer

Although immune gene signatures can stratify patients with ER-negative disease in terms of survival outcomes, the use of this information in clinical decision making is rather limited. Even in those patients classified as having a better prognosis, the number of relapses within 5 years remains sufficiently high to justify adjuvant chemotherapy. However, the interplay between tumor and immune system is complex because of the multiple opposing signals and feedback loops that coexist between various immune cells and cancer cells (Grivennikov et al. 2010). Therefore, subtypes of lymphocytes, macrophages, granulocytes, and antigen presenting cells may need to be considered separately when evaluating the prognostic and predictive value of the immune system. Specific metagene signatures for specialized T- and B-lymphocytes, and cells of the dendritic or macrophage/monocyte lineage have been used for this purpose (Rody et al. 2009, 2011; Schmidt et al. 2008; Bianchini et al. 2010b; Gu-Trantien et al. 2013). Similarly, large immunohistochemical studies with specific antibodies to track individual immune system components have also been performed (Karn et al. 2015). However, in most tumors co-infiltration by many different types of immune cells has been observed (Rody et al. 2009; Ruffell et al. 2012) resulting in high inter-correlation of all immune markers. Even markers linked to immunosuppressive activity, such as PD-1, PD-L1, CTLA4, show a significant positive correlation with other immune markers and with TILs (Denkert et al. 2015). These findings fit well with the intercorrelated nature of local immune biomarkers that may result from feedback loops between immune activation and suppression. Antithetical effects on prognosis have been observed for some types of immune cells, such as CD68+ and CD4+ cells, allowing their use as a combined prognostic score (Ruffell et al. 2012). Likewise, the combination of a B-cell metagene associated with good prognosis with the opposing effect of an IL-8 metagene resulted in a clinically relevant gene signature for triple-negative and basal-like breast cancer (Rody et al. 2011; Hanker et al. 2013). On the other hand modulation of T-cell response has demonstrated clinical efficacy in solid tumors (Topalian et al. 2012). Examples include new therapeutic antibodies that unleash the antitumor properties of the immune system effectively as ipilimumab, or antibodies that block PD1 (programmed cell death 1) and PD-L1 (programmed cell death 1 ligand 1) (Herbst et al. 2014). Current results allow monitoring potential antitumor immunity in breast cancer, but we are not yet able to reliably monitor the immunosuppressive activity in the tumor immune infiltrate. Therefore, the clinical utility of immune markers in ER-negative cancer still remains marginal, but may have a greater potential in combination with the upcoming immune therapeutic approaches.

19.7 Gene Mutations as Markers in ER-Negative Breast Cancer

An additional class of genomic markers are individual mutational changes within cancer genes. In general, two types of gene mutations can contribute to cancer. Somatic mutations that occur during lifetime and generate a founder cell of a cancer or a tumor subclone (Stratton 2011), as well as germline mutations in cancer predisposition genes, that are present in all cells and increase the risk of cancer (Rahman 2014b). Examples of the latter include the BRCA1 and BRCA2 genes. The benefits of determining whether a cancer is caused by a hereditary germline mutation could be undeniable (Rahman 2014b; Narod 2010). For patients it may provide

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better understanding of the genetic causes of their cancer and the higher cancer risk would justify prophylactic testing of other family members. It can also provide important information for disease management regarding surgery, radiotherapy, and chemotherapy (Narod 2010; Trainer et al. 2010). For example, platinum-based treatment is not standard for breast cancer but can have utility in BRCA mutation carriers (Byrski et al. 2012; Turner and Tutt 2012; Foulkes and Shuen 2013). Moreover, BRCA deficiency is the basis for the synthetic lethality approach exemplified by PARP inhibitors (Foulkes and Shuen 2013; Fong et al. 2009; Farmer et al. 2005a). Testing for BRCA1 mutations in patients with breast cancer has been referred to as medical genetic testing in contrast to predictive genetic testing aimed to estimate cancer risk in unaffected people (Rahman 2014a). BRCA1 mutation frequency of 2–3 % has been reported in women with breast cancer (Malone et al. 2006) but may increase to more than 10 % among younger patients with triple-negative disease (Narod 2010; Trainer et al. 2010). This highlights the importance of BRCA1 deficiency as a genomic marker in ER negative, and especially triple-negative breast cancer. With the advent of next generation sequencing (NGS) methods (Shendure and Ji 2008) faster and more affordable testing now allows eligibility criteria to be relaxed and results to be delivered within the timeframe required to impact cancer management (Rahman 2014a). Besides the BRCA genes, a handful of rare, highly penetrant genes, including TP53, PTEN, LKB1, as well as more frequent low penetrance genes, such as CHECK2, ATM, PALB, have been described as hereditary factors associated with breast cancer (Chung and Chanock 2011). However, a clinically useful genomic marker in breast cancer would require that the respective mutation affects patient prognosis or impacts her therapeutic management. In addition to cancer predisposing genes which may also have an impact on prognosis (Fasching et al. 2012) there is additional interest in the genetic background that could result in variation in drug-response phenotypes based on metabolism, transportation elimination affecting both efficacy and toxicity of a drug (Wang et al. 2011; McLeod 2013). Such germline DNA variants may help optimize cancer drug dosing and adverse side effects to improve benefit/risk ratio of cancer treatment. This field is referred to as pharmacogenetics or pharmacogenomics. Important examples of predictive factors regarding targeted treatment have been identified in other cancers, but no validated pharmacogenomic markers for ER-negative breast cancer are yet available since those studies involve major challenges which are currently beginning to be addressed (Wang et al. 2011; McLeod 2013).

19.8 Somatically Mutated Genes in ER-Negative Breast Cancer

As already addressed, the clinically most important somatically mutated gene and genomic marker in ER-negative breast cancer is the expression of HER2, altered mainly through gene amplification but also by activating mutations (Bose et al. 2013). Nevertheless, fueled by dramatic improvements in sequencing power and falling costs in the last decade, cancer genome sequencing projects have vastly increased our knowledge about the presence and frequency of somatic mutations in cancer. Such somatic mutations are identified by comparing tumor DNA with germline sequence obtained. e.g., from peripheral blood lymphocytes. Somatic mutations may be distinguished as either ‘driver’ mutations conferring a selective growth advantage to the cancer cells or ‘passenger’ mutations (Garraway and Lander 2013). Although this definition is simple in principle, it is more difficult to clearly identify, which somatic mutations belong into each category (Vogelstein et al. 2013). Passengers encompass all those neutral mutations that have been accumulated during normal development in the founder cell of the tumor, before the oncogenic event had occurred (Shibata 2012). These passenger mutations seem to account for roughly half of the mutations found in a typical breast cancer (Jones et al. 2008). A large part of the remaining mutations would also be passengers acquired after the

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tumor initiating event (Bozic et al. 2010). Individual genes can contain both driver mutations and passenger mutations. Thus the term "Mut-driver genes" has been coined to categorize genes suspected of increasing the selective growth advantage of tumor cells (Vogelstein et al. 2013). Although further cancer genome sequencing may unveil additional Mut-driver genes, the current data suggest that a plateau has being reached (Garraway and Lander 2013; Vogelstein et al. 2013). It has been estimated that for each tumor type about two thousand samples are needed to assemble the catalogue of coding mutations present in at least 2 % of tumors of a given type (Lawrence et al. 2014). For breast cancer more than half of that number has been profiled by The Cancer Genome Atlas (TCGA). Thus, at least for the coding sequence, substantial data are available on the frequency and distribution of mutations in breast cancer subtypes (Cancer Genome Atlas Network 2012; Stephens et al. 2012). The sobering perspective on the diversity is that driver mutations are operative in many cancer genes, but only a few are commonly mutated. Many infrequently mutated genes represent the long tail of the distribution, collectively making up a substantial contribution in myriad different combinations (Stephens et al. 2012). The number of genes frequently altered in breast cancers is rather low. Only three genes (PIK3CA, TP53, GATA3) were found to be mutated in at least 10 % of breast tumors and three additional genes in at least 5 % of the patients (Cancer Genome Atlas Network 2012; Stephens et al. 2012; Shah et al. 2012). However, the majority of the 20,000 detected somatic mutations in 500 breast cancers were observed only sporadically (Cancer Genome Atlas Network 2012; Stephens et al. 2012). It appears that virtually no two tumors have a similar mutational pattern (Karn 2013). Nevertheless, different mutations may be grouped to common oncogenic pathways somewhat reducing this complexity (Cancer Genome Atlas Network 2012; Stephens et al. 2012; Garraway and Lander 2013; Vogelstein et al. 2013; Hanahan and Weinberg 2011). TP53 is the most frequently mutated gene in ER-negative breast cancer, being mutated in about 80 % of basal-like tumors and in 92 % of ER-negative, HER2-enriched breast tumors (Cancer Genome Atlas Network 2012; Stephens et al. 2012). Unfortunately, however, TP53 currently does not represent a clinically "actionable" mutation in breast cancer. Several potentially targetable mutations (MAP3K1, MAP2K4, GATA3) are seen predominantly in ER-positive tumors. In 104 triple-negative tumors very few of the identified mutations were potentially druggable illustrating the challenges of developing new treatments and respective predictive markers for this subtype (Shah et al. 2012; Banerji et al. 2012). The frequency of PIK3CA mutations is the highest in luminal subtypes of breast cancer, but still considerable in ER-negative HER2-positive disease (Cancer Genome Atlas Network 2012). Because of the large amount of preclinical data available on activated PI3K pathway and resistance to HER2-targeted treatment, the role of this marker has been intensively studied. However, although differences in response to neoadjuvant therapy with different HER2-targeted treatments according to PIK3CA mutation status have been observed (Loibl et al. 2014; Majewski et al. 2015), these did not translate to significant clinical benefit in terms of improved overall or disease free survival (Pogue-Geile et al. 2015; Cescon and Bedard 2015). Thus, PIK3CA mutation testing is not a clinically useful test to guide treatment selection at the present time, but is should be incorporated in trials assessing the value of PI3K inhibitor combinations with HER2-targeted treatments (Cescon and Bedard 2015).

Access to next generation sequencing technology has recently spread out to basic translational research and clinical laboratories, and even if the throughput has not been adapted for high coverage genome sequencing projects, these systems are well suited for targeted sequencing of a smaller number of genes. Several cancer-specific gene panels have been introduced based on the assembled catalog of mutations from the recent cancer genome projects, and are being offered as high throughput genomic assays (Frampton et al. 2013). The clinical utility or actionability of the respective gene mutations as genomic markers

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partially depends on how "actionability" is defined; e.g., either in a broad prognostic sense or narrowly regarding prediction of response to specific drugs. Several institutional, regional, and global molecular screening programs that apply such gene panels have been launched with the intent to use this information to inform clinical decision-making (Hansen and Bedard 2013). These programs may provide enrichment strategies improving the likelihood of success for testing new cancer drugs. The true merits of this approach remain to be established. But in contrast to inefficient, sequential testing of rare alterations, such comprehensive testing of multiple biomarkers early in the course of disease together with access to a broad portfolio of matched investigational or approved drugs is most likely to advance personalized cancer medicine (Hansen and Bedard 2013). Even ultra-deep sequencing of such panels can be performed to detect rare subclones coping with the problem of tumor heterogeneity. Thus personalized tumor profiling may be feasible in a clinical setting ultimately translating genome sequencing from bench to bedside (Corless 2011).

19.9 Global Genome Alterations in ER-Negative Breast Cancer

Results from TCGA revealed that on average there are 57 (range, 5–374) mutations in the coding sequence of breast cancer (Cancer Genome Atlas Network 2012). ER-negative breast cancer displays a clearly higher mutational frequency with 1.94 nonsilent coding mutations per Mb of DNA compared to 1.35 in ER-positive tumors (Ng et al. 2015). Despite this higher mutational load, TP53 represents the single most recurrently mutated gene (84.5 %) in ER-negative tumors, in contrast to PIK3CA, GATA3, and MAP3K1 that are mutated more frequently in ER-positive tumors. In addition to somatic point mutations, cancers may also be characterized by structural DNA alterations such as deletions and copy number variations. Combining genomics, transcriptomics, and epigenomics has already provided novel insights, and new genome-driven integrated classifications of breast cancer that include DNA copy number changes have been proposed (Banerji et al. 2012; Curtis et al. 2012; Dawson et al. 2013). The TCGA breast cancer study used both SNP and CGH arrays, DNA methylation analysis as well as both transcriptome, proteome, and microRNA expression analysis to obtain comprehensive portraits of the molecular subtypes through integrative analysis across platforms (Cancer Genome Atlas Network 2012). This analysis revealed that in addition to loss TP53, loss of RB1 and BRCA1 as well as high MYC activation are basal-like features. The basal-like subtype moreover displayed similarity to high grade serous ovarian cancer, which is in line with the suggested value of PARP inhibitors and platinum compounds in both diseases. Thus, it is conceivable that future genomic markers for ER-negative breast cancer may also combine several complementary molecular features. Based on the dominance of either mutational changes or copy number alterations cancers may be categorized as M or C class. While about two third of ER-positive cancers seem to belong to the M class, literally all TNBC are of the C class type as are ovarian cancers (Ciriello et al. 2013). Whole genome sequencing of some tumors has also revealed massive genomic rearrangements acquired in single catastrophic events during cancer development (Stephens et al. 2011).

Markers for deficiency in homologous DNA recombination (HRD) are of great interest since they may predict response to PARP-inhibitors and to platinum based chemotherapy, as discussed above for BRCA1. Different markers have been developed to evaluate so-called genomic scars that remained in the tumor genome (Abkevich et al. 2012; Birkbak et al. 2012; Popova et al. 2012; Vollebergh et al. 2011; Wang et al. 2012; Watkins et al. 2015). Such signatures are associated with defects in error-free repair of interstrand crosslinks (Watkins et al. 2014). However, secondary events resulting in resistance to PARP inhibitors and DNA damaging chemotherapies limit the positive predictive value and clinical utility of these biomarkers (Watkins et al. 2014; Schouten and Linn 2015). In addition to therapies directed at HRD, other flaws in the genomic maintenance machinery that leave a detectable imprint in the genome and

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which may be targeted therapeutically could also become biomarkers. The large number of cancer genomes available has allowed identification of several mutational signatures giving further clues on the mutational processes shaping tumors (Alexandrov et al. 2013; Nik-Zainal et al. 2012). For example, Signature 6 of Alexandrov et al. was found to be associated with mismatch repair deficient cancers (Alexandrov et al. 2013).

Another important aspect has been observed through ultra-deep sequencing needed to establish the frequency of different subclones within the tumor. Such analyses have revealed extraordinary high intra-tumoral heterogeneity, especially in TNBC (Shah et al. 2012; Nik-Zainal et al. 2012). Those studies raised concerns that biomarker analyses from single biopsies may not cover the heterogeneous subclonality of tumors, thus ultimately leading to uncertainties in treatment decisions (McGranahan and Swanton 2015). For example tumor subclones resistant to single targeted treatments may preexist within the cancer at diagnosis. Consequently, this may suggest the need for multitarget approaches already at the start of therapy in order to eradicate the cancer (Vogelstein et al. 2013; Aparicio and Caldas 2013). On the other hand, however, the high mutational load in ER-negative breast cancer associated with this heterogeneity may be beneficial for the development of an immune response to the tumor (Rizvi et al. 2015; Le et al. 2015). In this respect mutational derived neoantigen load may form a biomarker for potential future immunotherapy of ER-negative breast cancer and provide an incentive for the development of novel therapeutic approaches that selectively enhance T cell reactivity against this class of antigens (Schumacher and Schreiber 2015).

19.10 Current Clinical Utility of Genomic Tests for ER-Negative Breast Cancer

The clinically most useful biomarker for ER-negative breast cancer is HER2 status. Unfortunately, the clinical utility of other available genomic tests for ER-negative breast cancer is currently still limited. The Ki67 score, a proliferation marker, post chemotherapy or the reduction of the score during neoadjuvant chemotherapy was not prognostic in TNBC (Balko et al. 2014). Furthermore, gene expression based commercially available prognostic tests have value mainly in ER-positive disease (Reis-Filho and Pusztai 2011; Gyorffy et al. 2015). Substratification of TNBC by gene expression, or integrated analyses including copy number alterations, allows to further distinguish subtypes with different prognosis and potential therapeutic targets. Still those classification systems may not yet be ready for prime time (Ng et al. 2015). Immune biomarkers are established and validated prognostic and predictive factors for both triple-negative and for HER2-positive breast cancers (Karn et al. 2015). They should be used as stratification tools in future clinical trials and several biological and therapeutic hypotheses can be formulated based on these associations. However, the clinical utility of immune parameters for informing decisions about standard adjuvant therapies for TNBC or HER2-positive cancers is currently limited. A very promising research direction is to explore the potential predictive value of immune cell infiltration for future immunotherapeutic regimens; e.g., as checkpoint inhibitors. Currently, among potential analyses of mutated genes only tests for BRCA1/2 have clinical utility regarding therapeutic decisions (Foulkes and Shuen 2013). PIK3A testing is not at present a clinically useful test to guide treatment selection in ER-negative disease (Cescon and Bedard 2015). Also, validated pharmacogenomic markers are not yet available for ER-negative breast cancer (McLeod 2013). Gene panel sequencing approaches combining comprehensive lists of genes found to be somatically mutated in tumors are currently under evaluation in several large studies. These may provide strategies for enrichment of cohorts for testing new drugs but their clinical utility has still to be established (Hansen and Bedard 2013). Several tests based on mutational scars in the genome as surrogates for DNA repair deficiencies have been developed and some of them are

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currently tested in clinical trials. However, final results for their use in clinical practice are not yet available (Schouten and Linn 2015).

19.11 Conclusions

One current and rapidly evolving topic in ER-negative breast cancer and in other solid tumors is the development of onco-immune therapies and the beginning understanding of the complex nature of the interface between tumor and host. It may be conceivable that a better understanding of these relationships may also provide new superior biomarkers for ER-negative breast cancers.

The recent developments in high throughput sequencing also suggest that this field may generate important novel genomic markers for cancer in general. Pilot studies have already shown that it is possible to analyze the complete genome of patients' tumors in a cost-effective and clinically relevant timeframe (Corless 2011). It is hoped that identified mutations may allow prediction of response to therapy with the ultimate aim of personalized cancer diagnostics (Corless 2011). Because of the infrequency of most alterations such methods would be germane to allow experimental "genome forward" trials or bucket trials for new therapeutics targeting such specific alterations (Bedard et al. 2013; Simon and Roychowdhury 2013). Whole genome sequencing data further suggest that each breast cancer has at least one DNA rearrangement. Thus, personalized cancer sequencing could lead to specific individual genomic markers which are suited for highly sensitive non-invasive disease monitoring by liquid biopsies (Aparicio and Caldas 2013). An important drawback for genomic markers may be the high heterogeneity and clonal diversity revealed by such methods, especially in ER-negative breast cancers (Shah et al. 2012; Nik-Zainal et al. 2012; Bedard et al. 2013). This can lead to both spatial and temporal heterogeneity within primary cancers and metastases posing questions about the value of single biopsies (McGranahan and Swanton 2015). Therefore, currently it is also far from clear how to define a threshold for an "actionable" alteration based on its subclonal frequency in the tumor (Ng et al. 2015), while on the other hand heterogeneity itself may also represent a biomarker (McGranahan and Swanton 2015). Furthermore, it is entirely possible that what constitutes a driver mutation is not universal but instead is cancer-specific. Inherited risk-modifying functional germline mutations could interact with somatic mutations appearing later to give rise to a founder cancer cell, whereas the same somatic mutation may be inactive in a different genetic background (Agarwal et al. 2015).

In conclusion, even when until now no new genomic markers in ER-negative breast cancers beside HER2 status have provided utility in clinical practice, their development is a constantly evolving topic. However, especially because of the poor prognosis of TNBC tremendous research efforts in this area are currently undertaken and may eventually result in the translation of clinically relevant biomarkers into the clinic. AQ1

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