Characterization of the mouse and human breast cancer genome

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Chapter 1

General Introduction
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Abstract

Recent advances in the characterization of different breast cancer types have intensified our focus on the discovery of druggable targets for the development of more selective breast cancer treatments. Breast cancer-related deaths have markedly decreased with the discovery of endocrine agents (e.g. tamoxifen and aromatase inhibitors) and trastuzumab that selectively target ER- and HER2-expressing breast tumors, respectively. This serves as a motivation to continue to define potential druggable targets for hormone receptor- and HER2-negative (triple-negative) breast cancers that do not respond to endocrine therapies or to trastuzumab and for which cytotoxic chemotherapy is the only systemic treatment option.

In-depth characterization of breast tumors is imperative for the development of targeted therapies. Here we review the phenotypic and genotypic characteristics of hereditary BRCA1- and BRCA2-mutated tumors relative to triple-negative breast cancers and sporadic breast tumors. We discuss differences in comparative genomic hybridization (aCGH) profiles, TP53 mutation status and compare these with characteristics of cognate BRCA1- and BRCA2-deficient mouse mammary tumors.

1. Introduction

Breast cancer is the most common malignancy in western women, who have a greater than 12.7% lifetime risk of developing this disease. In 2007, an estimated 1.3 million new cases were diagnosed worldwide, and 0.4 million of breast cancer patients died of the disease (1). Breast cancer incidence and death rates generally increase with age, illustrated by the fact that in the US, between 2002-2006, 95% of new breast cancer cases and 97% of breast cancer-related deaths occurred in women aged 40 and older (2). Improvements in early breast cancer detection and development of new treatment strategies have caused U.S. breast cancer deaths rates to decrease by 37% between 1990 and 2005 (3). Breast cancer treatment may involve local treatment (surgery, radiation therapy) alone or in combination with systemic treatment (chemotherapy or hormonal therapy). However, all of these systemic treatment strategies have unwanted side effects that can range from temporary infertility (in case of hormonal
treatments and chemotherapy) to toxic side effects of chemotherapy. In the past ten years it has become clear that there are several subtypes of breast cancer, each with their distinct biological features, clinical outcomes, and responses to chemotherapy. This new knowledge has led to the realization that not all breast cancers can be treated in the same way. This initiated the pursuit for treatment strategies based on individual tumor characteristics, so that the patient receives treatments to which her breast cancer will likely respond, without having to suffer from side-effects from treatments that are expected not to have an effect. The development of such tailored therapies for different tumor types depends on the identification of tumor characteristics that may constitute druggable targets.

2. Normal mammary gland morphology

The phenotypic diversity of breast cancers is closely linked to the morphology of the normal mammary gland and the molecular signals to which different types of normal mammary epithelial cells are exposed. In the normal mammary gland, lobules and ducts are lined with a double layer of epithelial cells: an inner layer of luminal cells and an outer layer of basal/myoepithelial cells that are in direct contact with the basement membrane. The morphology of the healthy breast is subject to continuous changes as a result of fluctuating plasma levels of ovarian sex hormones, such as estrogen and progesterone. These hormones influence the morphology of female reproductive organs in order to create a monthly chance for conception and subsequent pregnancy.

In the healthy, premenopausal mammary gland only 7%-15% of the epithelial cells express the estrogen receptor (ER) (4, 5). ER-positive cells often also express other hormone receptors, such as the progesterone (PR) and the prolactin (PrlR) receptors allowing response to changes in levels and ratios of hormonal signals. These hormone receptor positive cells exert paracrine signals to surrounding ER-negative cells (6-8), so that together they induce changes in mammary gland morphology corresponding with the phase of the estrous cycle (9-11). Interestingly, proliferating epithelial cells of the premenopausal breast rarely express hormone receptors, and conversely ER-positive cells only rarely divide (4, 12, 13).

Not much is known about differential proliferation rates of specific cells within the
postmenopausal mammary gland. When ovarian sex hormone production stops during menopause, the reproductive organs become atrophic. In the breast, this drop in plasma hormone levels causes a marked involution of the terminal ductal lobular units (TDLUs) (14). Interestingly, this process is accompanied by an increase in ER and PR expression in the epithelia of the residual ducts and small remnants leading to predominantly ER-positive postmenopausal breast tissue (14, 15). In conclusion, the fraction of hormone receptor expressing cells and overall gland morphology is completely different between the functional premenopausal breast gland and the atrophic postmenopausal breast gland.

3. Hormone receptor- and HER2-positive breast cancer

Clearly, ER and PR expression are important determinants of normal mammary epithelial cells, but to a great extent they also determine breast tumor characteristics and treatment choice. Approximately 60-80% of all breast tumors are ER-positive (16-19) which sensitizes them to treatment with systemic hormonal therapies such as tamoxifen and raloxifen, designed to inhibit estrogen signaling. Based on clinical testing, tamoxifen has a good risk benefit ratio in premenopausal women whereas raloxifene has a better safety profile in postmenopausal women (20). In addition, CYP19 aromatase inhibitors (AI) were developed to inhibit the conversion of androgens (produced in the adrenals) into estrogens by a process called aromatization (21, 22). AIs, such as anastrazole are effective in postmenopausal women, because they effectively inhibit the conversion of androgens into estrogens that occurs in peripheral tissues such as adipose tissue (23). AIs are not given to premenopausal women whose ovaries are still functioning because a temporary inhibition of estradiol production in the ovaries will raise gonadotropin levels which stimulates follicular growth and thereby estradiol production (24). Therefore, to inhibit follicular growth stimulation, premenopausal breast cancer patients are given the option to temporarily suppress ovarian function by injections with gonadotropin releasing hormone (GnRH) agonists (OFS) which, counterintuitively, inhibits gonadotropin function. However, the low estrogen levels caused by AIs and OFS can cause serious, sometimes intolerable side effects such as arthralgia (joint pain) and osteoporosis. Therefore, and because tamoxifen with OFS is at least as effective as anastrozole with OFS in premenopausal
patients (25), clinicians still prefer the use of tamoxifen over AIs in premenopausal patients. In postmenopausal patients, clinical attention is also starting to revert back to tamoxifen because, similar to premenopausal patients, side effects associated with tamoxifen are often better tolerated compared to those associated with AIs (26-28, Sabine Linn, personal communication). Although the optimal hormonal therapy is subject of debate among clinicians, it is has become clear that hormonal therapies are effective strategies to target ER-positive breast cancers. Indeed, their efficacy in the adjuvant and metastatic setting has contributed significantly to the reduction in breast cancer deaths over the last twenty years (3).

In 1985, the HER2 receptor, a member of the epidermal growth factor receptor (EGFR) family was found to be amplified and highly expressed in 20-30% of breast cancers (29, 30). This led to the development of trastuzumab, a HER2 targeting monoclonal antibody (31) which showed a remarkable beneficial effect when administered to patients with HER2 positive breast cancers both in the adjuvant setting (32-34) and the metastatic setting (35).

In the clinic, the availability of endocrine agents and HER2-targeting therapeutics has led to the routine practice to stain breast tumor sections for ER/PR and HER2 expression, in order to select the most promising systemic treatment. Its success recognized the importance of tumor characterization for prediction of therapy response to guide treatment choice.

4. **Triple-negative and basal-like breast cancer**

Approximately 15-20% of all breast tumors stain negative for ER, PR and HER2. These so called triple-negative breast cancers (TNBCs) do not respond to endocrine therapies or to trastuzumab. For patients with TNBC, cytotoxic chemotherapy is the only option for adjuvant treatment (36). Most ER-negative breast cancers, occur predominantly in premenopausal women and are associated with young age: the younger the woman, the greater the chance that her breast tumor is ER-negative or TNBC (17). Compared to other breast cancer patient groups, women with TNBC have a lower recurrence-free and overall survival, regardless of disease stage at time of diagnosis (37-39). However, patients with early stage TNBC have a decreased risk for a local relapse following locoregional radiotherapy and significantly higher
rates of pathological complete remission after neoadjuvant chemotherapy, indicating that these tumors are generally sensitive to these therapies (40-42). This sensitivity of the primary TNBC to neoadjuvant treatment with DNA-damaging agents and radiotherapy might be an indication of a specific feature of this tumor type. Identification of these specific features may allow targeting the metastatic TNBC in other ways than systemic treatment with cytotoxic chemotherapy (43).

Tumor classification by gene expression profiling has made a major contribution to the characterization of breast tumors. The landmark studies from Perou and Sorlie showed that breast cancers can be divided into five major molecular subtypes by linking histological features with hierarchical clustering of gene expression profiles (44-46). Tumors derived from a luminal cell type are mostly ER/PR-positive and CK8/18 positive and their expression profiles clustered into two separate branches, denoted Luminal A and Luminal B. Gene expression profiles of tumors overexpressing the HER2 receptor clustered in a separate “HER2-positive” branch. Similarly, expression profiles of breast tumors that are histologically very similar to normal breast tissue clustered in a separate “normal breast like” branch. Interestingly, TNBCs largely overlapped with tumors that clustered in a separate branch, which was termed “basal-like” because of the high expression levels of genes that are specific for basal/myoepithelial cells of the normal breast (47-49). These markers include basal cytokeratins (5/6, 14 and 17), p-cadherin and caveolin 1 (37, 45, 50-53) Historically, a subgroup of aggressive breast carcinomas showing features of myoepithelial/ basal differentiation was identified by histopathological methods (54-59).

Basal-like breast cancers (BLBCs) as determined by expression profiling show an approximate 70-80% overlap with TNBCs as determined by immunohistochemistry (IHC) (37, 60). Expression of basal markers CK5/6, CK17, CK14 and EGFR identifies a biologically and clinically distinct subgroup of TNBCs, justifying the use of basal markers in TNBCs to define BLBC (61). In support of this, a 5-marker BLBC group (ER/PR/HER2 negative, CK5/6 and EGFR positive) corresponded to the poor prognosis subgroup within the 3-marker (ER/PR/HER2 negative) TNBC group, highlighting the prognostic impact of the basal-like CK5/6 and EGFR markers (62). BLBCs often express both luminal and basal cytokeratins, suggesting that BLBCs may have more features of a dual-lineage differentiation phenotype than the subgroup of TNBCs that do not express basal cytokeratins and/or EGFR (43,
5. Hereditary breast cancers

Since cancer is a disease caused by genomic alterations, mutations in genes involved in safeguarding genomic integrity can confer an increased overall cancer risk. Indeed, increased breast cancer risk is associated with germline mutations in several genes involved in DNA repair mechanisms and cell cycle control: BRCA1, BRCA2, CHK2, ATM, NBS1, RAD50, BRIP1, PALB2, TP53 and PTEN (64, 65). Approximately half of all hereditary breast cancers are associated with heterozygous germline mutations in BRCA1 or BRCA2. Recent estimates of cumulative breast cancer risk at age 80 are 90% and 41% for BRCA1 and BRCA2 mutation carriers, respectively (66). Like the BLBCs, breast tumors from BRCA1-mutation carriers are associated with a young age of onset, with high tumor grade, with TP53 mutations and with the poorly differentiated basal-like phenotype (42, 67-70). Breast cancers of BRCA2-mutation carriers are also associated with a young age of onset, but to a lesser extent than BRCA1-related tumors (65, 71). In contrast to BRCA1-related breast cancers, BRCA2-related tumors are not of any specific tumor type. Therefore, although both BRCA1 and BRCA2 are involved in DNA repair, BRCA1- and BRCA2-related breast cancer show marked phenotypic differences. The exact reason for this difference is unclear, but may in part relate to the reported role of BRCA1 in mammary epithelial cell differentiation (72).

6. Molecular functions of BRCA1 and BRCA2

The BRCA1 gene was cloned in 1994 (73), and the BRCA2 gene a year later (74). Molecularly, the BRCA1 and BRCA2 proteins are very different: with its 3418 amino acids, BRCA2 is larger than BRCA1, which has 1863 amino acids. Although BRCA1 and BRCA2 are structurally unrelated, both proteins have been implicated in error-free DNA double-strand break (DSB) repair by homologous recombination (HR) (75, 76). Cells with non-functional BRCA1 or BRCA2 revert to error-prone DSB repair by non-homologous end joining (NHEJ) or single-strand annealing (SSA), resulting in genomic instability and ultimately to tumorigenesis (77). BRCA1
has also been implicated in cell cycle checkpoint (78), protein ubiquitination (79, 80) and chromatin remodeling (81-83).

In line with their structural differences, BRCA1 and BRCA2 have distinct functions in the DNA damage response (DDR) pathway. BRCA1 appears to be a DDR signal integrator that binds to many proteins and thereby targets them to the sites of DSBs. BRCA2, on the other hand, may play a more direct role in DSB repair by facilitating RAD51-mediated nucleoprotein filament formation on single-stranded DNA.

**Roles of BRCA1 and BRCA2 in the DDR pathway**

Following DNA damage, a DSB is recognized by the Mre11/RAD50/NBS1 (MRN) complex, which keeps adjacent DNA ends together before the repair process starts (84). The MRN complex recruits the ataxia telangiectasia mutated protein (ATM) which in turn phosphorylates BRCA1 (85, 86) and the histone H2AX in the chromatin domain flanking the DNA break, leading to more MRN and ATM binding, thus propagating the damage signal throughout the chromatin domain (87). The role of BRCA1 in the chromatin domain is not clear, but it might function as a signal for the activation of other components of the DDR machinery, or by modifying the chromatin structure surrounding the lesion in order to facilitate repair of DSBs (88).

At the site of the DSB, CtIP functions to regulate DSB end-processing and the generation of ssDNA which is avidly bound by RPA. ssDNA-RPA also recruits the ATM and Rad3-related protein kinase (ATR), triggering ATR-dependent checkpoint signaling by the protein kinase Chk1 (89). ATM and ATR activate the BRCA1-BARD1 complex by phosphorylating BRCA1 (85, 86, 90, 91), which is necessary for the formation of RAD51 filaments required for the DNA strand invasion and homology search steps associated with HR (92). A mediator complex including BRCA1-BARD1 and BRCA2-DSS1, probably bridged by PALB2 (partner and localizer of BRCA2) replaces RPA with RAD51 (93, 94). In this step, the RAD51 loading is provided by BRCA2, which interacts directly with RAD51 through its BRC repeats (75, 95-99). BRCA1 also interacts with RAD51 (95, 100, 101), but the exact nature of this interaction is unknown. However, many proteins involved in RAD51 function are products of hereditary cancer predisposition genes,
implying that the genomic instability resulting from failure to adequately regulate HR plays a causal role in cancer (64).

7. **BRCA1-related breast cancer and BLBC**

The great majority of breast tumors that occur in carriers of a heterozygous germ-line BRCA1-mutation share many phenotypic features with BLBCs: they stain negative for ER/PR and HER2 and express CK5/6 and CK14, CK17, and they are often TP53 mutated (45, 67, 102). Indeed, BRCA1-associated breast cancers and BLBCs have similar gene expression profiles (103). Like TNBC and BLBC, BRCA1-related tumors are associated with a young age of onset and early (<5 year) relapse, and both tumor types have a similar increase in pulmonary and brain metastases and reduction in bone metastases, compared to sporadic tumors (104, 105).

Since the vast majority of BRCA1-mutated breast cancers have a BLBC phenotype, BRCA1 dysfunction - or a related defect - could be a shared feature of both tumor groups. Indeed, recent studies have hinted towards a role for BRCA1 in the differentiation of mammary epithelial cells (106, 107). Depletion of BRCA1 up-regulates expression of genes involved in proliferation but down-regulates expression of differentiation-associated genes in a 3D cell culture system (106). Reduction of BRCA1 levels in human mammary epithelial cells led to an increase in stem cell-like cells and a reduction in ER-positive cells in vitro, and resulted in outgrowth of abnormal, undifferentiated structures in vivo, suggesting that loss of BRCA1 blocks luminal differentiation, leading to an increase in stem/progenitor cells (72).

8. **Chemosensitivity of BRCA1-mutated breast cancers and BLBCs**

Presumably due to the indispensable role of BRCA1 in DNA repair, BRCA1-deficient cells have been found to be extremely sensitive to DNA-damaging and DNA cross-linking agents such as cisplatin (108, 109). Preclinical studies in mouse models showed that also BRCA1-deficient tumors are highly sensitive to cisplatin (110). In line with this, a pilot study in patients with TNBC showed that neoadjuvant cisplatin therapy yielded a complete pathologic response in 9/10 breast tumors from BRCA1-carriers (111).
Another, more elegant therapeutic strategy to target HR-deficient cells makes use of the fact that BRCA1- and BRCA2-deficient cells are highly sensitive to inhibition of the base excision repair (BER) pathway, which results in accumulation of DSBs due to replication fork collapse at unrepaired SSBs. Whereas HR-proficient non-tumor cells can repair these DSBs by their intact HR-pathway, HR-deficient tumor cells can only utilize error-prone DSB repair pathways such as NHEJ and SSA, leading to gross chromosomal rearrangements and apoptosis or mitotic catastrophe. This synthetic lethal interaction between BRCA1/2 mutation and BER inactivation is the rationale behind the design of chemical inhibitors of poly(ADP-ribose) polymerase-1 (PARP1), a key enzyme in the BER pathway (112, 113). Indeed, the clinical PARP-inhibitor olaparib (AZD2281, from KuDOS-AstraZeneca) caused regression of BRCA1-deficient mammary tumors in mice (114). Furthermore, phase I and phase II clinical trials showed that olaparib as single agent has durable, objective antitumor activity in BRCA1- and BRCA2-mutation carriers with metastatic breast, ovarian or prostate cancer (115, 116). Another phase II study showed that TNBC patients treated with a different PARP inhibitor (BSI-201, BiPAR) in combination with standard gemcitabine and carboplatin chemotherapy had a significantly increased progression free survival compared to TNBC patients who received standard chemotherapy alone (117).

Together, these promising results suggest that platinum drugs and PARP inhibitors might not only be effective against BRCA1/2-mutated tumors but also against non-hereditary tumors with defects in HR. This raises the intriguing possibility that a subset of patients with non-hereditary BLBC might benefit from treatment with platinum drugs, alkylating agents or PARP inhibitors. Obviously, this requires careful selection of patients on the basis of yet to be developed biomarkers for HR deficiency.

9. TP53 mutations in sporadic and BRCA-associated breast cancer

Somatically acquired mutations in the TP53 gene occur in approximately half of all human cancers including breast cancers (118). TP53 mutation is a frequent event during malignant transformation because of its dual role in tumor suppression. TP53 (also known as transformation-related protein or tumor protein 53) can be activated
by p53-stabilizing protein ARF following oncogenic stress (119) or other stress signals such as membrane damage, oxidative stress, or osmotic shock. However, TP53 can also be activated by the DDR pathway kinases ATM/ATR and CHK1/CHK2, through direct phosphorylation or through destruction of the negative regulators MDM2 and MDM4 (120).

Small numbers of DNA breaks or single stranded gaps can already lead to activation of TP53 (121), which, depending on the severity of DNA damage, regulates gene expression needed to activate DNA repair, growth arrest or apoptosis pathways (122-124). For this reason, survival of genomically instable cells with BRCA1 or BRCA2 dysfunction cells could depend on abrogation of TP53 mediated apoptosis by TP53 mutation. Indeed, in mouse models, Trp53 mutation rescues the lethality of Brea1−/− and Brea2−/− embryos to a later developmental stage (72, 125-129). Moreover, Trp53 deletion markedly accelerates mammary tumor formation in mammary gland-specific Brea1 and Brea2 knockout mice (72, 128-130). In line with this, BRCA1- and BRCA2-mutated breast tumors are associated with an increased frequency of TP53 mutations (131-136). TP53 mutation is strongly associated with high grade, hormone receptor negative, basal like breast tumors and with increased global genomic instability (137-140) characteristic of BRCA1-related breast tumors and BLBCs (36, 141, 142). Furthermore, TP53 mutations have recently been associated with a poor prognosis in breast cancer patients (143) and squamous head and neck cancer patients (144) but also with increased sensitivity to high-dose chemotherapy or dose-dense epiurubicin-cyclophosphamide (145-147).

TP53 mutations reported in human tumors are commonly missense mutations that occur at hotspots in the DNA binding domain of TP53. Because active TP53 is a tetrameric complex of TP53 monomers, heterozygous TP53 mutations can result in the production of dominant-negative (DN) mutant TP53 proteins that bind and inactivate the wild-type TP53 protein encoded by the non-mutated allele. This gives rise to accumulation of mutant TP53 protein, which can be assessed by immunohistochemical staining with a TP53 antibody (148-150). In the clinic, this method has been commonly used to identify TP53 mutations in tumor tissues. An important limitation of this method is that it does not detect TP53 mutations that do not give rise to an accumulation of TP53 protein. As a result, the reported TP53 mutations in human cancers may be biased toward dominant-negative TP53
mutations. Indeed, the vast majority of TP53 mutations in the IARC TP53 database\(^1\) are hotspot mutations, with an especially high mutation frequency at residues Arg175, Arg248 and Arg273 (151).

Aside from dominant-negative TP53 mutations, TP53 mutation can lead to TP53 loss-of-function (LOF), which is frequent among missense mutants, but especially associated with truncating frameshift, splicing and nonsense mutations that lead to nonsense-mediated mRNA decay (152). Also, TP53 mutation can give rise to a TP53 protein with gain-of-function properties (GOF), which confers new functions to the mutant TP53 protein that are independent from wild type TP53 function. For example, TP53 mutants such as R175H, Y220C and R248W bind and inactivate TP53 family members p63 and p73, which are transcription factors involved in promoting apoptosis (153, 154). Furthermore, the D281G TP53 mutant with a functional transcription-activation domain has been suggested to activate MDR1 activation, leading to protection from drug induced apoptosis (155, 156). Although TP53 mutants have been shown to regulate several additional genes (157), a common mechanism for mutant TP53 regulated gene transcription is lacking, due to differences in TP53 mutations and effects (158).

Although in a large-scale study that analyzed 1,794 patients with breast cancer, found evidence for DN, LOF and GOF TP53 mutants, most TP53 mutations were missense mutations (142). However, a substantial fraction of breast cancers and head and neck cancers contain truncating TP53 mutations (136, 143, 144, 159, 160), which have been found to have a prognostic value similar to TP53 hotspot mutations (143). Although truncation mutations are commonly associated with loss of function, for each individual TP53 truncation mutation it remains to be established whether the truncated TP53 alleles are still expressed, or whether they can still exert residual TP53 functions or express a TP53 protein with a GOF.

Our finding that TP53 mutation occurs in almost all BRCA1-related and BLBCs, with a similar increase in truncating TP53 mutations could point to a strong selection for TP53 mutation in both the BLBCs and BRCA1-mutated breast tumors and perhaps to a common HR deficiency (136, 159) (Chapters 4 and 5 of this thesis). This common feature may (partly) explain the increased sensitivity to high-dose or

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\(^1\) http://www-p53.iarc.fr
dose-dense chemotherapy in TP53-mutated BLBCs and BRCA1-related breast tumors and BLBCs (145-147). In line with this, Bartz et al showed that silencing of BRCA1 enhanced cisplatin cytotoxicity approximately 4- to 7-fold more in TP53-deficient cells than in matched TP53 wild-type cells (161). Thus, tumor cells with combined disruptions in TP53 and BRCA1/2 network genes are more sensitive to cisplatin than cells with either disruption alone (161).

10. Comparative genomic hybridization analysis of breast cancer

The frequent occurrence of DNA copy number aberrations (CNAs) in breast cancers was recognized by conventional cytogenetics. However, the clinical implications of CNAs in breast cancer became clear with the finding that HER2/ERBB2 gene amplification, which occurs in 20-30% of human breast cancers, is associated with a poor clinical prognosis (162). The search for additional CNAs that could be markers for prediction or prognosis in cancers has led to the development of comparative genomic hybridization (CGH) and subsequently array-based CGH (aCGH) techniques to measure CNAs in sample (tumor) DNA relative to normal diploid DNA (163-165).

Initial aCGH studies showed that tumors from BRCA1- and BRCA2-mutation carriers have a greater degree of genomic instability compared with sporadic breast tumors, which is associated with their HR deficient phenotype (166-169). However, there are only minor differences between the genomic profiles of BRCA2-mutated breast tumors and sporadic breast tumors (167) (Chapter 3 of this thesis), which is in agreement with the overlapping phenotype of BRCA2-mutated and sporadic tumors (67, 170). Of note, aCGH analysis of 26 male breast cancers, including five tumors from BRCA2 mutation carriers, suggests that BRCA2-mutated tumors have more chromosomal aberrations than sporadic tumors and that, despite substantial hormonal differences, similar genetic changes are selected for during development of male and female breast cancer (168, 171).

Although the genomic aberrations in BRCA1-mutated breast tumors are distinctly different from those in luminal sporadic tumors (172), this aCGH profile is shared between BRCA1-mutated breast tumors and TNBC/BLBC (67, 170, 173, 174) (Chapter 5 of this thesis). This led to the idea that sporadic tumors with a BRCA1-
like aCGH profile might have an unknown *BRCA1* mutation or another mutation giving rise to genome instability. Previously, Wessels *et al.* generated a CGH classifier for identification of BRCA1-related breast tumors, based on chromosomal arms 3p, 3q and 5q. This classifier was able to predict the presence of *BRCA1* germline mutations with an accuracy of 84% (166). Indeed, using an optimized BRCA1 aCGH classifier, two of 48 breast tumors from patients with a family history of breast and ovarian cancer could be identified as BRCA1-associated tumors (172). Vollebergh *et al.* evaluated the BRCA1 aCGH classifier as a tool to effectively identify BRCA1-like sporadic tumors that respond to high-dose alkylating therapy (175). Excitingly, they found that patients with a BRCA1-like aCGH profile had a high complete remission-rate and long progression free survival after high-dose chemotherapy with carboplatin, thiotepa and cyclophosphamide.

One question remains: are there any specific BRCA1- or BRCA2- related genomic aberrations in *BRCA1*- or *BRCA2*-mutated tumors, or is the pattern of genomic aberrations mainly characterized by cell type? Comparison of the genomic profiles of hereditary *BRCA1*-mutated tumors with non-hereditary BLBCs and comparison of *BRCA2*-mutated tumors with sporadic breast cancers yielded only few BRCA1/2-specific genomic aberrations (Chapters 3 and 5 of this thesis). This suggests that the patterns of genomic aberrations selected for during tumor development are not markedly influenced by *BRCA1/2*-mutation. Rather, BRCA1/2 loss appears to facilitate development of DNA amplifications and deletions, leading to more intensified but otherwise similar patterns of genomic aberrations in *BRCA1*-mutated tumors vs. non-hereditary BLBCs and *BRCA2*-mutated tumors vs. sporadic breast cancers.

11. Cross-species oncogenomics to identify novel breast cancer genes and drug targets

Although the different types of human breast cancers have been analyzed extensively by gene expression profiling and by aCGH analysis, there has been limited progress in identifying the functional significance of recurrent CNAs in breast cancers, even when integrating both techniques (176, 177). Recurrent genomic aberrations usually
span large regions containing many genes, making it difficult to identify the gene that drives selection for the development of a gain or a loss during tumorigenesis. An additional difficulty is that genes that are amplified are not necessarily overexpressed (178, 179). However, when a gain or loss occurs at the same genomic region in multiple tumors, there must be an underlying selection pressure (180). A first step in the identification of the object of this selection pressure is to identify the minimal region of overlap of recurrent CNAs using computational methods such as GISTIC (181) or KC-SMART (182) (Chapter 2 of this thesis). Nonetheless, distinguishing unknown driver genes from passenger genes remains a major challenge. The molecular mechanisms that govern cancer-relevant processes such as cellular proliferation and survival are, however, conserved through evolution (183, 184). Therefore, aCGH data from tumors derived from genetically engineered mouse models can be used as a filter for identifying genes that represent strong candidates for a role in human cancer development (184). Indeed, several studies have compared mouse and human cognate cancers and this has allowed association of oncogenes with genomic aberrations. Cross-species comparisons of aCGH data from human and mouse neuroblastomas (185, 186), and similarly, epithelial ovarian cancers (187) yielded several conserved genetic aberrations. Likewise, comparison of aCGH profiles from mouse and human hepatocellular carcinomas identified Yap and cIAP1 to act synergistically as oncogenes in a syntenic focal amplicon (188). Also, metastatic cell lines from a mouse Ras-activated melanoma model contained an amplicon on the Nedd9 locus, which was overexpressed in human metastatic melanomas (189).

An important difference between mouse and human cancer development is that the engineered mutations in mouse models predispose to relatively rapid tumor formation, which circumvents the long incubation time required for accumulation of genomic mutations in human tumorigenesis. Indeed, most genetically engineered mouse models do not show the high levels of chromosome instability associated with human cancers. Therefore, “triple knockout” mouse models with combined deficiencies in p53, telomere maintenance and DNA damage signaling have been generated to produce mouse T-cell lymphomas with highly complex genomes (190). Cross-species comparison of aCGH profiles from these mouse lymphomas with profiles from human T-cell acute lymphoblastic leukemia/lymphoma (T-ALL) identified FBXW7 and PTEN to be commonly deleted both in mouse and human
tumors.

We have used a similar approach in an attempt to identify driver genes in the aCGH profiles from human BRCA1- and BRCA2-mutated breast cancers and cognate mouse Brca1Δ/Δ;Trp53Δ/Δ and Brca2Δ/Δ;Trp53Δ/Δ mammary tumors. We assumed that the genomic instability induced by BRCA1/2 loss in human and mouse mammary epithelial cells facilitates amplification and/or deletion of syntenic regions harboring orthologous driver genes. However, as with human BRCA1/2-deficient breast cancers, it is difficult to distinguish driver mutations from the background of passenger mutations in the genomically complex BRCA1/2-deficient mouse mammary tumors. Notwithstanding these difficulties, our cross-species comparison of mouse and human tumors identified several evolutionarily conserved loci and genes involved in the development of BRCA1- and BRCA2-related breast cancer (Chapter 7 of this thesis).

12. Conclusions

To be successful in designing better, safer, more effective and more individualized treatments for patients with BRCA1- or BRCA2-mutated breast cancers it is imperative to identify features that are characteristic for BRCA1- or BRCA2-mutated tumors, and which could serve as possible druggable targets.

Both in mice and in humans, BRCA1-mutated breast cancers skew towards a basal-like phenotype. In contrast, the cellular phenotypes of both mouse Brca2Δ/Δ;Trp53Δ/Δ tumors and human BRCA2-mutated tumors are more heterogeneous and resemble those of sporadic human breast tumors or Trp53−/− mouse mammary tumors. Other than in DNA repair, BRCA1 is involved in several other cellular processes, one of which could underlie the formation of undifferentiated, basal-like breast tumors with an aggressive phenotype. These tumors do not respond to endocrine or trastuzumab treatment due to the lack of hormone receptors or HER2 expression. In-depth characterization of BRCA1-mutated breast tumors has revealed an intimate link between BLBC and BRCA1-related breast tumors by their common TP53 mutation status and aCGH profile. Indeed, TP53-deficiency may be a prerequisite and thus a hallmark for HR-deficient tumors which, together, could explain the similarities in tumor grade, proliferative index and chemosensitivity between BLBCs, BRCA1-
mutated tumors and TP53-deficient breast cancers.

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