Characterization of the mouse and human breast cancer genome

Holstege, H.

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: https://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.

UvA-DARE is a service provided by the library of the University of Amsterdam (http://dare.uva.nl)
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 breast cancer patients died of the disease. Like all cancers, breast cancer is not only caused by point mutations and small insertions/deletions but also by genomic rearrangements such as translocations, regional deletions/amplifications or by gain or loss of whole chromosome arms. These genomic rearrangements can cause alterations in cellular proliferation and survival pathways, which can result in unscheduled cell growth, invasion of surrounding tissues and ultimately metastatic cancer. Therefore, it makes sense to analyze tumors for recurrent DNA rearrangements and the impact this might have on gene expression, subsequent protein expression and cell functionality. DNA copy number changes are assessed with comparative genome hybridization (CGH), in which the copy numbers of specific sequences of the tumor DNA are compared with DNA of normal tissue. With the development of array-based CGH (aCGH) technology it has become possible to analyze in a single experiment and with high resolution the entire genome for DNA copy number alterations (CNAs). Genomic loci that are repeatedly mutated during cancer development, i.e. that occur in multiple independent tumors, might harbor genes important for tumor development. Identification of new cancer genes could lead to the identification of novel candidate drug targets. To identify these recurrent CNAs, we have developed a new method, KC-SMART, which analyzes aCGH data from a group of tumors and detects CNAs that occur significantly more often within the group than expected by chance (Chapter 2).

Since cancer development is driven by genomic alterations, mutations in genes involved in safeguarding genomic integrity can confer an increased overall cancer risk. Indeed, carriers of germline mutations in specific genes involved in DNA repair mechanisms and cell cycle control have an increased risk of developing breast cancer.
Approximately half of all hereditary breast cancers involves *BRCA1* or *BRCA2* mutations. Recent estimates of breast-cancer risk by the age of 80 years are 90% for *BRCA1*-mutation carriers and 41% for *BRCA2*-mutation carriers. Both BRCA1 and BRCA2 have indispensable functions in homologous recombination (HR) repair, an error-free DNA repair mechanism to fix double-strand breaks (DSB). Cells with non-functional BRCA1 or BRCA2 revert to error-prone non-homologous end joining (NHEJ) or single-strand annealing (SSA), resulting in genomic instability and ultimately tumorigenesis. Breast cancers from *BRCA1*-mutation carriers are associated with a young age of onset, with high tumor grade, with *TP53* mutations and with an undifferentiated basal-like phenotype. Breast cancers of *BRCA2*-mutation carriers are also associated with a young age of onset, but to a lesser extent than *BRCA1*-mutation carriers. In contrast to *BRCA1*-mutated tumors, *BRCA2*-mutated breast cancers are not of any specific tumor type.

We compared the genomic aberrations from tumors in *BRCA1*- and *BRCA2*-mutation carriers with a set of non-hereditary (sporadic) breast tumors. To detect differences in recurrent CNAs in *BRCA1*- or *BRCA2*-mutated tumors vs. sporadic tumors, we developed *comparative-KC-SMART*, an algorithm that detects recurrent CNAs that occur more often in one tumor group compared to another group (Chapter 3). Using this approach, we found that aCGH profiles of *BRCA1*-mutated tumors were markedly different from those of *BRCA2*-mutated tumors, which resembled sporadic tumors. The differentially recurrent CNAs in these tumor groups could represent driver mutations that specifically collaborate with either *BRCA1*- or *BRCA2* loss-of-function in tumorigenesis. Alternatively, they might harbor cancer genes that are specific for malignant transformation of basal vs. luminal mammary epithelial cells.

Somatic *TP53* mutations occur in approximately half of all human cancers including breast cancers. The TP53 protein is an essential tumor suppressor: if DNA gets damaged or if overexpression of oncogenes drives unscheduled cell proliferation, wild type TP53 is activated and can initiate programmed cell death, apoptosis. By preventing sustenance of a mutated genome, TP53 prevents tumor development. For this reason, there is a strong selection pressure to mutate *TP53* during tumor development. The most common *TP53* mutations are so called “hotspot mutations”,
missense mutations that mostly reside in the TP53 DNA binding domain and cause aberrant TP53 folding. Basal-like breast cancers (BLBC), a subgroup of breast cancers that does not express the estrogen-receptor (ER), the progesterone receptor (PR) or the HER2 receptor, are associated with an undifferentiated phenotype and with a high frequency of TP53 mutations. Interestingly, we have found that all BRCA1-mutated tumors, which are HR deficient and have a BLBC phenotype, are TP53 mutated, whereas approximately half of all sporadic tumors is TP53 mutated. Moreover, we found that this increase of TP53 mutations was due to a specific increase in protein-truncating TP53 mutations. This suggests that survival of HR deficient cells depends on TP53 mutation, or that the genomic instability caused by HR deficiency causes an increase in insertions and deletions in the TP53 gene (Chapter 4).

Intriguingly, we found that the majority of non-hereditary BLBCs were also TP53 mutated, which was again due to an increase in truncating TP53 mutations (Chapter 5). In case the HR deficiency of BRCA1-mutated tumors is responsible for the occurrence of truncated TP53 mutations, then it is likely that sporadic BLBCs with truncated TP53 mutations are also HR defective. If HR deficiency does not underlie the increase in truncated TP53 mutations, then it is possible that the increased selection pressure for TP53 mutation in undifferentiated basal mammary epithelial cells might allow for more extreme TP53 mutations.

In order to obtain better, safer, more effective and more individualized treatments for cancer it is important to invest in the discovery of new cancer genes which can serve as potential druggable targets. Because BRCA1- and BRCA2-mutation give rise to an increased risk of breast cancer, mouse models with targeted mutations in these genes could be ultimate tools to study the specific genomic and phenotypic changes required for breast cancer development in vivo. For this reason we have developed conditional mouse models for BRCA1-mutated breast cancer (Chapter 6) and BRCA2-mutated breast cancer. These mouse models have tissue-specific deletion of Brca1 or Brca2 in combination with Trp53 deletion in epithelial tissues including mammary gland and develop “spontaneous” Brca1Δ/Δ;p53Δ/Δ and Brca2Δ/Δ;p53Δ/Δ mammary tumors. Interestingly, both mouse and human BRCA1-mutated tumors skew towards basal-like carcinomas suggesting that BRCA1 loss but not BRCA2
loss may block cellular differentiation as suggested previously, or alternatively, that BRCA1 loss is only tolerated in undifferentiated, basal mammary epithelial cells.

We found that Brca1 or Brca2 loss in combination with Trp53 loss aggravated the abnormalities in the aCGH profile of Trp53 tumors, but also gave rise to certain Brca1 and Brca2 specific CNAs. We have used aCGH data from these mammary tumors as a filter for identifying genes that represent strong candidates for a role in human BRCA1- and BRCA2-related breast cancer development. The phenotypic resemblance of mouse and human BRCA1- and BRCA2-mutated tumors is only partly reflected by the genotypic resemblance. We find some important similarities between aCGH profiles of mouse and human cognate cancers, such as the MYC gain and the RB/INTS6 loss, but also important differences. These differences might be due to the fact that tumor development in our mouse models is a fast, accelerated version of the relatively slow process of breast cancer development in humans. Also, besides differences in genomic organization, differences in mouse and human mammary gland biology may result in selection of other regions and genes during mammary tumorigenesis in both species. These differences should be taken into account when using mouse models for preclinical studies (Chapter 7).