Novel treatment strategies for hereditary breast cancer
Evers, B.

Citation for published version (APA):
English Summary
Every year, over 1.1 million patients are diagnosed with breast cancer. An important fraction of patients will die as a result of metastatic disease because curative therapeutics are not available for advanced breast cancer. In addition, certain treatments, such as mastectomy, have major social and psychological consequences calling for the development of less invasive approaches.

The population of breast cancer patients can be roughly divided in a group with a strong familiar background and a second group of patients in which hereditary predisposition seems to be absent, the so-called sporadic cases. Intensive research has pinpointed mutations in several genes to be responsible for the hereditary predisposition and led to the identification of the breast cancer susceptibility genes \textit{BRCA1} and \textit{BRCA2}.

The discovery of \textit{BRCA1} and \textit{BRCA2} sparked much research directed at elucidating the function of both genes in healthy cells and in the suppression of mammary tumorigenesis. Animal models for \textit{BRCA}-deficiency have significantly contributed to our current understanding of \textit{BRCA} function and Chapter 1 presents a comprehensive overview of the insights that these various models have offered. In part inspired by the close resemblance of the phenotype of \textit{BRCA}-deficient animals to other knock-out mice, researchers investigated and confirmed a role for both \textit{BRCA1} and \textit{BRCA2} in the faithful repair of DNA double-strand breaks (DSBs) through the process of homologous recombination.

While patients carry heterozygous \textit{BRCA} germline mutations, tumors of \textit{BRCA} mutation carriers have usually lost the wild-type allele. The resulting homologous recombination deficient (HRD) phenotype has thus been proposed to be the Achilles’ heel of \textit{BRCA}-deficient cancer. Indeed, cells with non-functional \textit{BRCA} were shown to be extraordinarily sensitive to DSB inducing agents. To test whether this sensitivity is also present in \textit{BRCA2}-deficient tumor cells, in which the HRD phenotype might be suppressed by additional mutations, we report the isolation of the first \textit{BRCA2}-deficient mouse mammary tumor cell lines in Chapter 2. Indeed, these cell lines were sensitive to the DSB inducing drug cisplatin, and in Chapter 3 we show this process to be dependent on \textit{BRCA2} expression.

Not only cisplatin, which directly modifies the DNA, was shown to induce differential toxicity in \textit{BRCA2}-deficient cells versus reconstituted controls. In fact, Olaparib (AZD2281), a novel inhibitor of Poly-(ADP-Ribose)-Polymerase (PARP) enzymatic activity, was shown to have the largest therapeutic index of all agents tested. PARP inhibitors are thought to indirectly cause DSBs through replication fork collapse at single-strand breaks, the repair of which is dependent on PARP activity.

Platinum containing drugs as well as PARP inhibitors are currently in clinical trials for treating \textit{BRCA}-deficient breast cancer, but it is unknown whether these compounds are most suitable for this aim. We therefore performed two pharmaceutical screens to identify novel compounds with specific activity against \textit{BRCA2}-deficient mammary tumor cells. The first screen, described in Chapter 3, was performed using a library containing drugs already known to the clinic. Three bi-functional alkylators, not currently used for treating breast cancer patients, were identified. Follow-up validation in mice harboring \textit{BRCA2}-deficient mammary tumors confirmed anti-tumor activity, which was as good as or even stronger than cisplatin and Olaparib. \textit{In vitro} combina-
tion studies showed synergism between these alkylators and Olaparib. In vivo, this synergy is recapitulated in some instances but needs further validation.

In the second pharmaceutical screen, described in Chapter 4, we tested 22,720 compounds with unknown activity for specific cytotoxicity against BRCA2-deficient tumor cells. Two compounds mediated strong toxicity in BRCA2-deficient tumor cells compared to wild-type cells. These compounds induce DSBs but are not PARP inhibitors. Further experiments should elucidate the exact mechanism of action of these agents.

During evolution, cells acquired many mechanisms that regulate cell growth and prevent tumorigenesis. Abrogation of just one such mechanism is thought to be insufficient for malignant transformation, which may depend on multiple (epi)genetic mutations and other cooperating events. For that reason, it is very important to not only develop animal models for single perturbations in oncogenic pathways, but to also study cancer gene collaborations and interactions between epithelial cells and the surrounding stroma. This type of research, however, is still very time-consuming and labor-intensive. Chapter 5 describes a novel mouse model for E-cadherin and p53 deficient mammary tumors, which allows for relative fast testing of the influence that additional mutations in the epithelial cells and/or modifications in the tumor stroma have on primary tumor development and metastasis formation. It is expected that a similar strategy can also be used to investigate cooperating mutations and tumor-stroma interactions of BRCA-associated mammary tumors.

In recent years, the concept of breast cancer stem cells has received much attention. Since the development of many tissues seems to originate from a very small compartment of multipotent cells, it has been postulated that disturbed homeostasis in this compartment could be causal to tumorigenesis. Since BMI1 is known to be essential for maintenance of hematopoietic and neural stem cells and is found overexpressed in human breast tumors, we investigated the role of BMI1 in normal mammary gland development. In Chapter 6 we present evidence that BMI1 is involved in mammary epithelial stem cell activity, but in addition plays a role in the differentiation program of more committed mammary epithelial cells.