Novel treatment strategies for hereditary breast cancer
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Chapter 7

General discussion
Breast cancer is the most important cause of neoplastic death in women worldwide (WHO, World Cancer Report 2008). It is a dreadful disease, hugely impacting the patient’s quality of life and her surroundings. Although the introduction of chemotherapy treatment has reduced mortality amongst breast cancer patients, major improvements are still required. Good treatment strategies have a high therapeutic index, meaning they specifically target certain unique characteristics of tumor cells that are not shared with the surrounding healthy cells. Therefore, understanding the processes underlying tumor formation and maintenance is crucial to the development of new therapeutics.

In the case of hereditary breast cancer, significant advances have been made in understanding the tumor etiology. Heterozygous mutations in the DNA damage repair genes BRCA1 and BRCA2 have been found to be responsible for a large fraction of hereditary cancers. Since tumors arising in BRCA mutation carriers have often lost the wild-type allele, BRCA-deficiency is a tumor-cell specific trait in these patients, allowing the development of targeted therapies.

1. DNA damaging agents for the treatment of hereditary breast cancer

The homologous recombination deficient (HRD) phenotype of BRCA-mutated tumors is currently being exploited in clinical trials. Both platinum containing agents, which directly induce DNA damage, and inhibitors of Poly-(ADP-Ribose) Polymerase (PARP) activity, which indirectly generate recombinogenic lesions, are being tested in BRCA patients who failed first-line treatment. Interestingly, the current standard of care for first-line treatment already contains doxorubicin and cyclophosphamide, both DNA damage inducing drugs. It will therefore be extremely interesting to test the new compounds in the preoperative setting to assess their performance relative to the current standard of care. Our results show that, in addition to platinum drugs and PARP inhibitors, alkylating agents such as chlorambucil, melphalan and nimustine may constitute attractive alternatives to inducing DNA damage. To achieve the highest therapeutic index, compounds should be used that induce precisely those types of DNA lesions that cannot be repaired in the absence of BRCA1 or BRCA2. This implies not only the necessity for determining the whole spectrum of lesions induced by any compound, but also the need for a complete picture of the relative contributions of different pathways to the repair of these lesions. In addition, activity of DNA repair pathways is known to be cell-cycle regulated. While non-homologous end-joining (NHEJ) is the preferred pathway in the G1 phase of the cell-cycle, replication-associated DSBs are repaired by HR. Thus, irradiation, which induces DSBs in all phases of the cell cycle, may prove less useful than agents inducing lesions that are converted to DSBs upon replication fork stalling.

2. Sporadic HRD tumors

Apart from BRCA-associated hereditary breast cancers, a considerable fraction of hormone receptor- and HER2-negative sporadic breast tumors (so-called “triple-negative” tumors) are also believed to be HRD tumors with BRCA-like characteristics. It can be expected that these tumors will have similar responses to DNA damaging agents as BRCA1/2 deficient tumors. Improvements in the detection of sporadic HRD tumors are needed, however. Diagnostic tests for identification of HRD tumors may lead to a significant benefit when patients are treated with agents targeting this tumor-specific phenotype. In addition, while BRCA-patients that failed first-line therapy are rare and thus difficult
to recruit for clinical trials, triple-negative patients with recurrent tumors may well be included in a Phase II trial testing the efficacy of melphalan or chlorambucil, especially when BRCA-like HRD tumors can be identified by e.g. array CGH based classification (Vollebergh et al., submitted and 15,16).

3. Tumor recurrences
Treating HRD tumors with cisplatin, AZD2281 or alkylating agents clearly seems a promising therapeutic strategy. Still, animal models have demonstrated tumor recurrences in studies using single-agent regimes and/or combinations with Olaparib (Chapter 3, 17,18). Clinically, these recurrences are important and a multitude of approaches should be taken to understand and ultimately prevent the emergence of tumor relapses.

3.1. Understanding and counteracting recurrence mechanisms
Efforts should be taken to understand the mechanisms underlying tumor relapses. Retreatment of recurring tumors should elucidate whether these acquired resistance against the drug of interest, or whether a subset of tumor cells can escape treatment. Examples of both exist and can be dealt with in several ways. For example, overexpression of the P-glycoprotein (P-gp) multidrug resistance transporter genes Abcb1a and Abcb1b was associated with acquired resistance to Olaparib, which could be counteracted by co-administration of tariquidar, an efficient inhibitor of P-gp activity18. Resistance to nimustine, which is not a substrate for P-gp19 is acquired through upregulation of O6-methylguanine-DNA methyltransferase (MGMT), an enzyme directly reverting the alkylation damage20. Thus, co-administration of compounds that lead to MGMT degradation, such as O6-methylguanine or O6-benzylguanine, may re-sensitize tumor cells to nimustine21.

BRCA1/2 deficient mammary tumors are highly sensitive to platinum-based drugs and recurring tumors remain sensitive to platinum treatment17. Nevertheless, tumors cannot be eradicated. It is possible that a subset of cells in the primary tumor stop cycling, preventing cisplatin mediated toxicity. Methods to force such cells into the cell-cycle are currently under investigation.

3.2. Dosing schedules
Alkylating agents not only induces DNA damage in the tumor cells, but also in other cells in the body. The toxicity that this damage induces in the hematopoietic system is often the dose-limiting factor. Nevertheless, since dose intensity was shown to be important for response to chemotherapy22, therapies were developed using high doses of alkylating agents followed by transplantation of autologous hematopoietic stem cells to reconstitute the damaged hematopoietic system23. This approach has been abandoned by many physicians due to uncertainties about the actual efficacy24. Recently, it was shown that sporadic tumors with a CGH profile similar to BRCA1 tumors responded very well to high-dose alkylating therapy (Vollebergh et al., submitted). This leads to the speculation that tumors with a BRCA1-like CGH profile are indeed HR defective, and that this HR-deficiency identifies patients benefiting from high-dose alkylating therapy.

For conventional chemotherapeutics, such as alkylators and platinum drugs, dose-response correlations have only been established in unselected populations of breast cancer patients. It is possible, however, that these correlations differ between HR deficient and HR proficient breast cancers, since normal cells are able to efficiently repair small amounts of DNA damage, whereas even small amounts of DSBs cannot be
resolved in HRD tumor cells. Hence, slow accumulation of DNA damage induced by metronomic chemotherapy may prove as toxic to HRD tumors as single high-dose therapy, while being much less toxic to the rest of the body. It may therefore be interesting to test the efficacy of long-term low-dose regimes against HRD tumors.

In conclusion, several interesting challenges lie ahead to determine the optimal dosing strategy of alkylating agents in HRD-deficient tumors. Animal models are crucial to testing several of these dosing strategies before the initiation of clinical trials.

3.3. Combination therapies

Besides combinations of alkylators or cisplatin with Olaparib - which we have shown to be synergistic in in vitro experiments - combinations of multiple alkylators with differential activity towards BRCA2-deficient cells may also prove useful. Different compounds may have non-overlapping resistance and toxicity mechanisms, while impinging on the same weaknesses of HRD tumor cells, thereby creating an even wider clinical window. The BRCA2 mammary tumor model used in the studies described in chapter 3 provides ample opportunity to test such combinations.

3.4. Novel targeted therapeutics

All compounds currently known to induce specific toxicity in BRCA deficient cells directly or indirectly induce DNA damage. Identifying additional DNA damaging agents may prove useful, especially when these have a favorable toxicity profile and/or prove to be less prone to resistance. However, the therapeutic window of targeting DSB repair deficiency may simply be not wide enough to lead to complete eradication of HRD tumors. It will therefore be relevant to identify additional synthetic lethal interactions in BRCA-deficient tumor cells. Performing siRNA based knock-down screens in our BRCA2-deficient tumor cells and isogenic reconstituted controls would be an obvious approach to reach that goal.

3.5. Targeting genetic revertants

BRCA2-mutated tumor cell lines and primary tumors with acquired resistance to PARP inhibitor or platinum drugs were recently shown to have acquired secondary mutations in BRCA2, restoring its function. Because restoring mutations make these tumors resistant against any strategy directed against BRCA2-deficiency, it will be relevant to develop alternative therapeutic strategies targeting characteristics that are specific to the etiology of BRCA2-deficient tumorigenesis. Unfortunately, our knowledge on additional pathway perturbations in BRCA2-associated tumors is limited. Since BRCA2 deficiency in primary cells is associated with a proliferation defect, accompanied by activation of the p53 pathway, it has been suggested that inactivation of this pathway is necessary for BRCA-associated tumorigenesis. Indeed, p53 deficiency accelerates BRCA2-associated tumorigenesis in mouse models and p53 is often mutated in human tumor samples of both BRCA1 and BRCA2 patients. Inhibition of CHK1, a protein involved in the S/G2 checkpoint after DNA damage is thought to be synthetically lethal with p53 deficiency. Especially because BRCA2 deficient cells are expected to accumulate large amounts of endogenous damage, inhibition of Chk1 in p53 deficient BRCA2 tumors may form an attractive additional approach.

Abrogation of the p53 pathway is probably not sufficient to overcome the proliferative arrest induced by BRCA loss, given that p53 inactivation only partially rescues BRCA2-associated embryonic lethality in mice. Experiments aimed at elucidating pathway perturbations that are able to fully
overcome the proliferative arrest in BRCA2-deficient cells, in the presence or absence of functional p53, hold great promise and may uncover additional therapeutic targets.

4. Novel mouse models to study BRCA-associated tumorigenesis
Both the genetic rescue screens in BRCA2-deficient primary cells and the synthetic lethal RNAi screens in BRCA2-deficient tumor cells will result in hits that need further validation. As reviewed in chapter 1, animal models are indispensable for this purpose. In chapter 5, we have proposed a novel mouse model that can be applied to test the contribution of additional hits to E-Cadherin and p53 mediated mammary tumor formation and progression. Similarly, it can be expected that transplantation of primary mouse mammary epithelial cells (MMECs) from K14cre;Brca1<sup>F/F</sup>;p53<sup>F/F</sup> and K14cre;Brca2<sup>F/F</sup>;p53<sup>F/F</sup> mice gives rise to tumors in the recipients that resemble those found in the donor mice and that tumor initiation is preceded by normal organogenesis. Such models can be used for in vivo validation of hits identified in the in vitro screens by genetic manipulation of primary MMECs prior to transplantation.

In addition, efforts are ongoing to create animal models with protein-truncating or missense mutations in Brca1 and Brca2 mimicking well-known pathogenic mutations in the human genes. Tumors occurring in these models are expected to be able to acquire resistance to platinum agents by genetic reversion. If this is the case, these models are very well suited to test therapeutic approaches targeting other aspects of BRCA-associated tumors.

5. Tumor stem cells in sporadic tumors
All the above mentioned strategies are geared specifically towards treating hereditary breast cancer patients, who constitute only a minority of all breast cancer patients. It is therefore important to gain further insight in the processes underlying sporadic tumorigenesis. In recent years, the tumor stem cell hypothesis has attracted attention from many investigators. This hypothesis postulates that only a small subset of all tumor cells is capable of both self renewal and complete tumor reconstitution<sup>34</sup>. Clinically, this has important implications that may shift therapeutic focus from killing the bulk of the tumor, consisting of highly proliferative non-stem cells, to specifically eliminating the subset of stem cells.

The existence of a stem cell responsible for normal mammary organogenesis has been reported recently<sup>35,36</sup> and deregulation of homeostatic processes in this cell compartment might create tumor stem-cells leading to full-blown tumors<sup>37</sup>. In chapter 6, we have shown a role for Bmi1 in the maintenance of the mammary stem cell population. Likewise, deregulation of Bmi1 activity in the mammary stem cell compartment might lead to unscheduled stem cell proliferation and subsequent tumorigenesis. This hypothesis remains to be tested by transplantation of cells overexpressing Bmi1. Approaches as described in chapter 5, using primary cells transduced with viruses mediating inducible Bmi1 overexpression, not only allow testing the tumorigenicity of Bmi1 overexpression, but also the potential of Bmi1 as a therapeutic target.

6. Integration of knowledge: Personalized combinatory medicine
Contemporary researchers often focus their attention on a very small portion of the multitude of complex biological systems underlying both healthy and diseased tis-
sue behavior. Many anti-cancer therapeutic strategies based on the results of these investigations have seen the light in the past decades with mixed success. Given the complexity of the cancer cell-intrinsic and –extrinsic oncogenic signaling networks, targeted monotherapy is unlikely to be the complete solution for any type of cancer. This is likely caused by both the intrinsic plasticity of many tumors and the strong heterogeneity between tumors. Specific properties rendering one tumor susceptible to a certain treatment may be absent in another and vice versa. Developing optimal combinations of anti-cancer drugs, each successfully targeting individual tumor characteristics, seems the inevitable way towards personalized medicine in oncology. Hopefully, the future will show that the research in this thesis has made a contribution to such tumor-tailored combination therapy.

References


