RAD51 as biomarker for the identification of homologous recombination deficient gynaecological carcinomas

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Chapter 1

Introduction

Gynaecological carcinomas and homologous recombination deficiency

Gynaecological carcinomas (ovarian, endometrial, cervical, vaginal, vulvar and breast) are among the ones leading to the highest cancer-related deaths worldwide [1]. The presence of germline pathogenic variants (gPV) in the breast cancer susceptibility genes *BRCA1* and *BRCA2* is associated with an increased risk for breast and ovarian cancer [2] and other cancers [3]. In 3% of all breast cancer (BC) patients, and in 10-15% of patients with triple-negative BC (TNBC), gPVs in *BRCA1/2* are observed [4]. Similarly, 15% of patients with ovarian cancer (OC) harbor gPVs in *BRCA1/2* and up to 30% of patients with high-grade serous OC (HGSOC) [5,6]. The BRCA proteins play a crucial role in the homologous recombination (HR) pathway, which is the only DNA damage repair pathway that can repair DNA double-strand breaks (DSBs) error-free. DNA damage occurs naturally every day in our cells, due to both endogenous (e.g. intracellular metabolism, DNA replication) and exogenous (e.g. UV-light and chemotherapeutic agents) factors [7,8]. The repair of DNA damage by HR is crucial to maintain genomic integrity. *BRCA1/2* deficiency, but also (epi) genetic defects in other HR-related genes or other unknown factors can lead to HR-Deficiency (HRD) [9]. Patients with HRD tumors are particularly sensitive to platinum-based chemotherapy and poly-ADP ribose polymerase inhibitors (PARPi) [10,11]. Consequently, patients with *BRCA1/2* deficient or HRD tumors are now eligible to receive PARPi targeted therapy [12].

PARPi therapy

Although our molecular understanding of tumorigenesis has advanced a lot in the last decades, treatment for patients with gynaecological carcinomas still largely involves the classical triad surgery, radiation therapy, chemotherapy and in some cases also targeted therapy. With the arrival of the targeted therapy with PARPi in 2010, new opportunities arrived for patients with *BRCA1/2* deficient or HRD ovarian cancer, and since recently also for patients with HER2-negative *BRCA1/2* deficient breast cancer, patients with *BRCA1/2* deficient prostate cancer and for patients with *BRCA1/2* deficient pancreatic cancer [13]. An inherited defect in either one of the *BRCA1/2* genes, or, in the case of ovarian cancer, another somatically acquired defect that leads to HRD is thus a prerequisite of receiving PARPi. However, more patients are likely to be HRD and could benefit from PARPi therapy, and current research focuses on identifying those patients.

HRD as biomarker

Besides *BRCA1/2* gPV carriers, another way to identify patients who could benefit from platinum and/or PARPi treatment, is by using HRD as biomarker. HRD tumors rely on other, error-prone DNA repair pathways for the repair of DNA damage, which leads to

genomic instability [14]. This results in certain genomic and mutational patterns in the DNA of the tumor cells, which can be identified by next-generation sequencing (NGS) methods. The so called DNA-based HRD tests use this approach to identify HRD tumors beyond *BRCA1/2* deficiency. Similarly, functional HRD tests are developed that determine the ability of tumor cells to perform HR by evaluation of the accumulation of RAD51 protein at sites of DNA damage [15-20]. The advantage of functional HRD tests over DNA-based HRD tests is that functional HRD tests determine the HR status real-time (at surgical removal), while DNA-based HRD tests reveal all historic mutational or DNA-damaging events that occurred during tumor development, which may not necessarily reflect the current HR status. *BRCA1/2* deficiency served as a gold standard for the development of both DNA-based and functional HRD tests. (Pre)clinical validation of a wide range of HRD tests is currently ongoing to explore the ability of such tests to identify patients with *BRCA1/2* deficiency, to validate test results with the results from other HRD tests, and to allow a better identification of all patients who are eligible for platinum/PARPi treatment and improve response rates to these treatments.

Aims and scope of this thesis

The aims of this thesis were:

- To develop a RAD51-based functional HRD test.
- To benchmark the RAD51-based HRD test with tumors harboring pathogenic variants in *BRCA1/2*.
- To benchmark the RAD51-based HRD test with other DNA-based HRD tests.
- To explore the presence of functional HRD among a variety of gynaecological carcinomas.

Chapter 2 provides a review on HRD, the currently available HRD tests, the pros and cons of the different methodologies, their sensitivity for the identification of *BRCA1/2* deficient and HRD tumors, their concordance with other HRD tests, and their capacity to predict therapy response.

Chapter 3 presents the functional analysis of HRD in 49 consecutively included ovarian carcinoma samples analyzed by the REcombination CAPacity (RECAP) test, by *ex vivo* irradiation of fresh tumor tissue. HRD was solely identified among high-grade serous ovarian carcinomas, including but not limited to tumors with *BRCA1/2* deficiency. A trend towards better overall survival of high-grade serous ovarian carcinoma patients with RECAP-identified HRD tumors compared to patients with HRP tumors was observed.

Chapter 4 describes the development and calibration of a functional RAD51-based assay on formalin-fixed paraffin-embedded diagnostic samples (RAD51-FFPE test). Twenty-five endometrial and 49 ovarian carcinomas were successfully analyzed by the RAD51-FFPE test. *BRCA1/2* deficient tumors and RECAP-HRD tumors were identified with a high sensitivity. Optimal RAD51-FFPE test parameters were defined for the identification of HRD among both types of carcinomas.

Chapter 5 highlights the relevance of HRD testing in endometrial carcinomas. Eighty endometrial carcinoma samples were analyzed by both the RAD51-FFPE test and RECAP test and HRD was commonly identified among high-grade serous, p53abn endometrial carcinomas. In addition, cervical and vulvar carcinoma samples were analyzed by the RECAP test, among which no HRD cases were identified.

Chapter 6 reports on the functional analysis of HRD in 63 breast carcinomas of various histological subtypes by using both the RECAP and the RAD51-FFPE test. Using matching tumor samples, the RAD51-FFPE test showed a high sensitivity to determine the HR-class as defined by the RECAP test with the previously defined test parameters for endometrial and ovarian carcinomas, confirming that these parameters are also suitable for the identification of HRD among breast carcinomas.

Chapter 7 shows the evaluation of the RAD51-FFPE test as an informative biomarker for HRD in a cohort of 249 triple-negative breast carcinomas (SCAN-B cohort). This study was sufficiently powered to compare the performance of the RAD51-FFPE test with a Genomic Scar test on the one hand, and the mutational signature test HRDetect on the other. In addition, RAD51-FFPE scores were related to the presence of (epi)genetic defects in HR-related genes. The RAD51-FFPE test identified tumors with (epi)genetic defects in HR and tumors with an HRD status as determined by the HRDetect and Genomic Scar DNA-based HRD tests with high sensitivity.

Chapter 8 discusses the findings of the conducted investigations in view of the latest literature and focuses on the future perspective of RAD51 as biomarker for the identification of HRD carcinomas.

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