The Fanconi anemia/BRCA2 pathway in pancreatic cancer
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Citation for published version (APA):
CHAPTER 3

SCOPE OF THE THESIS

As noted in chapter 2, approximately 4-7% of pancreatic cancer patients harbor germline mutations in the BRCA2 gene. In 2002, just prior to the start of this work, Howlett et al.\(^\text{42}\) found biallelic mutations in BRCA2 to be responsible for a subset of Fanconi anemia (FA) patients. FA is a hereditary cancer susceptibility disorder, with the occurrence of hematological abnormalities or acute myelogenous leukemia at an early age, usually leading to death before the age of 20. Patients who survive into adulthood often develop solid tumors, especially squamous cell carcinomas of the head and neck or the anogenital region\(^\text{45}\). The FA syndrome can be classified in at least 11 (genetically distinct) complementation groups\(^\text{102}\); the FANCA, FANCB, FANCC, FANCD1 (BRCA2), FANCD2, FANCE, FANCF, FANCG and FANCL genes have been cloned. The proteins encoded by the FA genes FANCA, FANCB, FANCC, FANCE, FANCF, FANCG and FANCL form a nuclear complex that is necessary for the monoubiquitination of Fancd2, the central protein in this pathway\(^\text{45-47}\). After the discovery of mutations in the BRCA2 gene in patients with FA, we investigated whether other FA genes could also be mutated in pancreatic cancer, perhaps leading to an increased susceptibility of a subset of pancreatic cancers to DNA-interstrand crosslinking agents. Most tumor-suppressor genes and caretaker genes in (pancreatic) cancer are inactivated by a mutation in one allele and loss of the other allele (loss of heterozygosity, LOH), or by loss of both alleles (homozygous deletion). Therefore, we first establish which of the xenografted pancreatic adenocarcinomas in our database have allelic loss at genomic locations dispersed over all chromosomes. For each tumor, loss of heterozygosity was assessed using 382 microsatellite markers with an average distance of 10 cM. Results are reported in chapter 4\(^\text{29}\).

The genomic locations 9q22 and 9p13, the locations of the FANCC and FANCG genes, respectively, had allelic loss in a large percentage of the pancreatic adenocarcinoma xenografts in our database. Therefore, we studied 22 pancreatic xenografts, selected for LOH, and 11 pancreatic cancer cell lines, for genetic mutations in the FANCC and FANCG genes.
(chapter 5)\(^{44}\). Two convincing mutations were found: a germline nonsense mutation in \(\textit{FANCC}\) in a cell line and a somatic frameshift mutation in \(\textit{FANCC}\) in a xenograft, both accompanied by loss of the second allele. A defect in Fancd2 monoubiquitination, which can be detected by a western blot, indicates that one of the upstream FA genes is defective. A total of twenty-one pancreatic cancer cell lines (including the 11 cell lines already sequenced) were screened using Fancd2 monoubiquitination as a functional assay. In this screen, another pancreatic cancer cell line was found to be defective in the FA pathway, which led to the discovery of a large homozygous deletion in \(\textit{FANCC}\) in this cell line (chapter 6)\(^{105}\). FA defective cell lines had an increased sensitivity to mitomycin C (MMC) and cisplatinum, as compared to the sensitivity of FA-proficient pancreatic cancer cell lines to these drugs. Patients who harbor 'sporadic' tumors defective in the FA pathway are not themselves hypersensitive to interstrand crosslinking agents. Therefore, the occurrence of FA-defective tumors in FA-proficient hosts could provide a very useful therapeutical window for the treatment with DNA-interstrand crosslinking agents, such as mitomycin C or cisplatin.

In chapter 7, we studied the effect of FA deficiencies on in vitro sensitivity to a large panel of commonly used chemotherapeutic agents and in vivo sensitivity to MMC, cyclophosphamide (a commonly used bifunctional alkylating agent) and gemcitabine (an anti-metabolite often used in the clinical treatment of pancreatic cancer). Using the previously described \(\textit{FANCC}\) and \(\textit{FANCG}\) defective cell lines, two pairs of genetically identical cell lines, solely varying in the presence or absence of one FA gene, were created. A stable transduction was established with either the cDNA of \(\textit{FANCC}\) or \(\textit{FANCG}\), or an empty vector. These cell line pairs allowed us to study the effect of FA defects on chemosensitivity to various drugs, with a minimum of confounding effects of genetic background. In chapter 8, we investigated whether germline mutations in the \(\textit{FANCC}\) and \(\textit{FANCG}\) genes could be present in patients with a strong family history of pancreatic cancer.

In chapter 9, we screened a panel of 35 non-pancreatic cancer cell lines for defects in the proximal FA pathway by Fancd2 immunoblot. In chapter 10, we studied mismatch repair deficient gastrointestinal tumors for defects in the \(\textit{BRCA2}\) and \(\textit{MRE11}\) genes. Statistical analysis of the mutation rates in these tumors suggests that defects in \(\textit{BRCA2}\) and \(\textit{MRE11}\) are not selected for in mismatch repair deficient tumors.