SUMMARY

Fanconi anemia (FA) is a rare genomic instability syndrome characterized by a variety of congenital malformations, bone marrow failure and cancer predisposition. The majority of FA patients develops bone marrow failure during the first decade of life, which has been a major cause of death. Since bone marrow transplantation outcomes have improved considerably in recent years, the next life-threatening problem FA patients are facing is the high chance of developing solid tumors, most particularly of the head and neck region. At the cellular level, FA cells are defective in repairing DNA damage induced by DNA interstrand crosslinking (ICL) agents, such as the widely used chemotherapeutic agent cisplatin. As a consequence of deficient DNA repair, FA cells exhibit increased genomic instability, which can lead to cancer. A better understanding of the FA proteins involved in this DNA repair pathway will provide insights into the mechanisms of ICL repair and malignant transformation as well as in chemotherapy response as FA cells are hypersensitive to the chemotherapeutic agent cisplatin. Therefore, part of the research described in this thesis focused on the identification of additional FA genes and the encoded proteins. Given the high susceptibility of FA patients to develop head and neck cancer, we also examined if errors in FA proteins occur in head and neck tumors of non-FA patients. Finally, we investigated if defects in the FA repair mechanism can be exploited in anti-cancer therapies.

In the first chapters of this thesis (**Chapter 2-4**), we describe the identification of two genes (*FANCP/SLX4* and *FANCQ/ERCC4/XPF*) that cause FA when mutated. Bi-allelic mutations in *FANCP/SLX4* were found in one Dutch and four German FA patients, whereas bi-allelic mutations in *FANCQ/ERCC4/XPF* were causative for FA in one German and one Spanish individual. Currently, 17 FA genes have been identified and the corresponding proteins function in the FA pathway to repair ICLs. The SLX4 protein plays a role in the coordination of several structure-specific endonucleases, including XPF, which are able to cut the DNA thereby initiating the removal of the ICL. XPF also functions in another DNA repair pathway, which is involved in repairing UV-induced DNA damage. Mutations in *FANCQ/ERCC4/XPF* have previously been identified in two other syndromes: Xeroderma pigmentosum (XP) and XPF-ERCC1 (XFE) progeroid syndrome. XP is characterized by increased sensitivity to UV light (sun light) and an associated enhanced risk to develop skin cancer, while the only reported XFE progeroid syndrome patient had a very severe phenotype with characteristics of both FA and XP. Thus, mutations in one gene (*XPF)* are now associated with three clinically different diseases: XP, XFE progeroid syndrome and FA. Depending on the type of mutation in *FANCQ/ERCC4/XPF*, one or both of the two DNA repair mechanisms in which XPF is involved are affected and this dictates the distinct clinical outcomes. In FA patients, the repair of ICLs is mainly affected, while the repair of UV-induced DNA damage is primarily defective in XP patients. When both repair mechanisms are deficient by mutations in *FANCQ/*

ERCC4/XPF, individuals will present with XFE progeroid syndrome.

In **Chapter 5**, we describe the occurrence of pathogenic mutations in FA genes in head and neck squamous cell carcinoma (HNSCC) cell lines derived from individuals without FA. Although we showed that a large number (53%) of these HNSCC cell lines had a typical FA feature (ICL-induced chromosomal breakage), the occurrence of FA gene defects in these cell lines was rare. Within panels of 17 and 39 HNSCC cell lines, we found one cell line with bi-allelic mutations in *FANCM* and one with promotor hypermethylation and loss of expression of *FANCF*. Inactivation of FA genes by mutations or promotor methylation leads to defective DNA repair and can result in ICL-induced chromosomal breakage and chromosomal instability (CIN), which is a hallmark of many types of cancer. Another possibility that can be causative of CIN is the unequal distribution of chromosomes to daughter cells when a cell divides. Chromosome segregation is regulated by several processes including sister chromatid cohesion and inherited defects in this process are associated with diseases collectively called cohesinopathies (*e.g.* Roberts syndrome and Warsaw Breakage syndrome). Since cells derived from Roberts syndrome or Warsaw breakage syndrome patients resemble cells from FA patients in terms of ICL-induced chromosomal breakage, we also studied the occurrence of sister chromatid cohesion defects in HNSCC cell lines. Severe sister chromatid cohesion defects were observed in 29% of HNSCC cell lines, which could be explained by mutations in *PDS5A* in one cell line and in *STAG2* in another cell line. Thus, inactivation of FA genes or defective sister chromatid cohesion occasionally occur in HNSCC cell lines, providing possible explanations for the observed CIN in a subset of head and neck tumors. Screening of tumor samples for these mutations might be of relevance to predict response to the chemotherapeutic agent cisplatin as FA deficient cells are hypersensitive to this drug. In many cases, however, we could not identify the responsible mutated gene that might explain the described cellular phenotypes.

In **Chapter 6**, we provide evidence that FA and sister chromatid cohesion defects might be exploited in anti-cancer therapies. Previous studies showed that FANCD1/BRCA2, FANCN/PALB2, FANCO/RAD51C or FANCP/SLX4 deficiency results in sensitivity to inhibitors of the protein PARP, and that this might be used in the treatment of cancer. We confirmed these results by demonstrating that lymphoblasts derived from FA patients with mutations in *FANCD1/BRCA2* and *FANCN/PALB2* were sensitive to PARP inhibitors as expected. However, FANCP/ SLX4-deficient lymphoblasts were not particularly sensitive to inhibition of PARP, which could be explained by the presence of truncated SLX4 protein with a residual function in these cells. We newly identified FANCM deficiency as a determinant of PARP inhibitor response as lymphoblasts with *FANCM* mutations were sensitive to inhibition of PARP. We also showed that the level of sensitivity of *FANCD1/BRCA2* mutant cells to PARP inhibition depended on the type of mutation. To test whether

cells derived from individuals with syndromes clinically related to FA also exhibited PARP inhibitor sensitivity, lymphoblasts of individuals with a cohesinopathy were also tested. Cells with mutations in *DDX11*, which is causative for Warsaw breakage syndrome, were also sensitive to PARP inhibition. Hence, we newly identified FANCM and DDX11 as determinants of PARP inhibitor response, which possibly extends the utility of these agents in the treatment of cancer.

To find additional targets that might be exploited to develop new anti-cancer therapies for tumors in FA patients as well as FA-deficient tumors in individuals without FA, we have performed a genome-wide high-throughput siRNA screen (see **Chapter 7**). Although more research is required, we found two classes of synthetic lethal interactions. The first class is tumor specific and independent of FA status, which thereby may provide a cancer treatment strategy in patients with or without FA. This class includes inhibition of the vacuolar ATPase, a proton pump involved in pH homeostasis, and the spindle assembly checkpoint, which is required for the correct distribution of chromosomes during cell division. The second class is FA-specific and is particularly promising to further exploit to develop anti-cancer treatments for the small group of patients that have a tumor with a defect in the FA pathway. This class includes inhibition of several nucleoporins (proteins that are part of a large multi-subunit complex involved in among others protein transport, gene expression and DNA repair) and the proteasome, which has an important role in protein degradation.

Overall the work presented in this thesis has led to the identification of two FA genes (*FANCP/SLX4* and *FANCQ/ERCC4/XPF*), the demonstration of rare known FA defects in head and neck tumor cell lines of non-FA individuals and the first steps in investigating whether these defects in tumors can be exploited to develop new anti-cancer treatment strategies.