CHAPTER 8

Discussion

Fanconi anemia (FA) is a rare genomic instability syndrome characterized by a plethora of congenital malformations, bone marrow failure and a high risk to develop cancer, in particular acute myeloid leukemia and squamous cell carcinomas of the head and neck region[1]. Given the remarkable sensitivity of FA cells to a specific group of chemotherapeutic drugs (i.e. DNA interstrand crosslinking (ICL) agents)[2,3], many studies have been performed to unravel the molecular mechanism that explains this phenotype. This led to insights into chemotherapy response and the discovery of 17 currently known FA genes (see also **Chapter 1-4**), of which the encoding proteins function in the FA pathway to repair DNA crosslinks[4–8]. Although our knowledge of FA is increasing, it is still not clear why FA patients specifically develop head and neck tumors and whether the inactivation of the FA pathway is involved in the etiology of head and neck squamous cell carcinoma (HNSCC) in non-FA individuals. In this thesis, we addressed this last question by examining the occurrence of FA pathway inactivation in cell lines of head and neck tumors of individuals without FA. Moreover, we also investigated whether FA defects can be exploited as a target in cancer therapy.

The extremely high risk to develop head and neck cancer in FA patients[9], suggests that inactivation of the FA pathway drives carcinogenesis. The finding that HNSCC cell lines derived from FA patients were indistinguishable from those derived from non-FA patients in terms of whole-arm translocations and numerous gains and losses[10,11], may suggest that a deficient FA pathway also drives carcinogenesis in squamous cells of the head and neck region in non-FA patients. In agreement with this, we showed in **Chapter 5** that more than half (53%) of the sporadic HNSCC cell lines tested have a typical FA feature: sensitivity to crosslinking agents in terms of ICL-induced chromosomal breakage. However, we also found that inactivation of the FA pathway is rare in sporadic HNSCC (**Chapter 5**), and only a few sequence variants in some FA genes (i.e. *BRCA1*, *BRCA2*, *FANCM* and *SLX4*) were found by whole exome sequencing of large groups of head and neck tumors[12,13]. Based upon these results, we think it is unlikely that FA pathway inactivation is a frequent driver of carcinogenesis in non-FA patients, albeit FA-like features frequently occur.

Since the occurrence of FA defects is rare, other mechanisms may be involved in chromosomal instability in sporadic HNSCC. Of note, ICL-induced chromosomal breakage is not exclusively a hallmark of FA cells and has been observed in lymphoblasts or T lymphocytes from individuals with other diseases, such as Nijmegen breakage syndrome, Roberts syndrome or Warsaw breakage syndrome[14–18]. The last two syndromes (belonging to a group of diseases collectively known as cohesinopathies) are caused by mutations in *ESCO2* or *DDX11*, respectively[17,19]. Cells derived from Roberts or Warsaw breakage syndrome patients are in addition to ICL sensitivity, characterized by sister chromatid cohesion defects (premature separation of chromatids during mitosis)[17–20]. Severe sister chromatid cohesion

defects were also observed in 5 HNSCC cell lines (**Chapter 5**). In one of these cell lines, the severe sister chromatid cohesion defect could be explained by mutations in *STAG2*, whereas in another cell line with less severe cohesion defects mutations in *PDS5A* were found. Whether inactivation of these genes results in increased ICL-induced chromosomal breakage should be further tested by functional studies. Notably, inactivation of STAG2 has been observed in several tumor types[21]. One study showed that STAG2 knockdown in a pancreatic adenocarcinoma cell line increased sensitivity to the ICL-inducing chemotherapeutic agent cisplatin[22], while another study showed that STAG2-deficiency was associated with PARP inhibitor sensitivity in glioblastoma cells[23]. These results suggest that tumors with defective sister chromatid cohesion may respond well to treatment with cisplatin or PARP inhibitor. Considering the potential to exploit sister chromatid cohesion defects as a target in anti-cancer therapy in HNSCC, further research is required to 1) verify that tumors with defective sister chromatid cohesion have a favorable response to cisplatin treatment (or chemoradiation) and 2) if this is the case, it would be relevant to determine whether screening for sequence variants in genes involved in sister chromatid cohesion predict response.

The observed ICL-induced chromosomal breakage may also be caused by overexpression of oncogenes, such as cyclin E, viral oncogene E7 and CDC25A. Overexpression of these oncogenes accelerates progression from G1 to S phase of the cell cycle, resulting in oncogene-induced replication stress (recently defined as slowing or stalling of replication fork progression and/or DNA synthesis[24]) and has been found associated with the formation of DNA double strand breaks[25–28]. Oncogene-induced replication stress activates the DNA damage response, which is an important barrier against malignant transformation in precancerous lesions. Stalling or slowing down of replication forks is likely due to limiting nucleotide concentrations[29], increased DNA torsional stress[30], the interference between transcription and DNA replication[31] or exhausted RPA protein levels[32]. As a consequence, replication forks collapse and massive chromosomal breakage occurs upon a persistent G2/M arrest or premature mitotic entry[33]. Likewise, increased DNA damage due to defective ICL-repair results in enhanced replication stress and consequently FA-deficient cells are persistently arrested in G2/M with extensive chromosomal breakage. Interestingly, some of the ICL-sensitive HNSCC cell lines in terms of ICL-induced chromosomal breakage did not arrest in the G2/M phase of the cell cycle upon treatment with ICL agents, indicating that the cells continue growing in the presence of DNA damage. The absence of an ICL-induced G2/M arrest may lie in a defective G2/M phase checkpoint, resulting in premature mitosis. This in combination with elevated replication stress due to overexpression of oncogenes and increased DNA damage caused by ICL-inducing agents may explain the observed ICL-induced chromosomal breakage in these cell lines. Thus, other mechanisms

such as sister chromatid cohesion defects and oncogene-induced replication stress in combination with a G2/M checkpoint defect might be responsible for the observed ICL-induced chromosomal breakage in sporadic HNSCC cell lines.

Mutations in the tumor suppressor gene *TP53* and loss of *CDKN2A* ($p16^{Ink4a}$) are the earliest and most frequently detectable genetic alteration in HNSCC, which can already be detected in premalignant dysplastic lesions[34]. These changes are also found in HNSCCs of FA patients, suggesting that FA pathway inactivation accelerates the typical squamous cell carcinogenesis process, causing frequent HNSCCs at young age in FA patients. In response to DNA damage, p53 coordinates several signaling pathways to maintain genomic stability: it activates DNA repair, causes cell cycle arrest to allow enough time to repair the damage or, in certain instances, initiates apoptosis[35]. Inactivation of the FA pathway can result in unrepaired DNA damage, leading to the activation of p53. This elevated p53 response has indeed been demonstrated in hematopoietic stem and progenitor cells from FA patients and *Fancd2*$^{-/-}$ and *Fancg*$^{-/-}$ mice[36]. Consequently, the primary bone marrow cells are arrested in the G1 phase of the cell cycle, which might eventually lead to hematopoietic stem cell depletion, thereby explaining the progressive impairment of hematopoiesis in FA. Besides providing insights in the pathogenesis of bone marrow failure, a hyperactive p53 response might also explain the increased cancer proneness of FA patients. Due to an overactive p53 DNA damage response, FA deficient cells may have a growth disadvantage due to increased cell cycle arrest and/or apoptosis. This would be alleviated in rare cells that have gained a *TP53* pathway mutation. Although deletion of p53 enables cells to tolerate DNA damage and rescues the defects of FA human and mouse hematopoietic progenitors[36], it will also result in enhanced genomic instability and tumor formation. Indeed, loss of p53 accelerated tumor formation in *Fancd2*$^{-/-}$ and *Fancc*$^{-/-}$ mice[37,38]. Likewise, it might also explain why individuals with the telomere maintenance disease Dyskeratosis congenita have an increased risk to develop HNSCC as shortening of telomeres will also result in elevated p53 activation[39–41]. Taken together, an exacerbated p53 response due to unresolved DNA damage might trigger rapid selection for *TP53* mutations in FA patients, driving tumorigenesis. In contrast, inactivation of the FA pathway before loss of p53 in a cell in a non-FA individual will result in an adequate DNA damage response, p53 activation and cell cycle arrest, which will prevent malignant transformation. For this reason, the occurrence of FA pathway defects in sporadic HNSCC might be rare.

Notwithstanding, FA-deficient tumors do occur occasionally in non-FA individuals. These tumors may respond well to chemoradiation, making it of significant interest to find biomarkers of FA-deficiency to predict treatment response and thereby personalize treatment. Since chemoradiation can cause many side effects, FA-deficiency might be further exploited in the treatment of cancer, which we

have shown in **Chapter 6** by demonstrating that in addition to BRCA1/2- or PALB2-deficiency, FANCM-deficient cells were sensitive to PARP inhibitors. Moreover, in **Chapter 7**, we noticed that more siRNAs were lethal in FANCC-deficient tumor cells compared to the corresponding FA-corrected tumor cells, indicating that synthetic lethal interactions may exist between FA deficiency and other pathways. Confirming the synthetic lethal interactions and understanding the underlying mechanism, will open new avenues in the treatment of FA-deficient tumors.

References

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