Chapter 7

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Discussion

"*Keep your head up, we are nearly there"*

Strange Colour Blue – Madrugada, 1999

Discussion and Future Prospects

DNA damage, mutations and genomic instability are established driving forces of cancer and other age-related diseases. Mutations in tumor suppressor genes and oncogenes are very frequently found in tumors and genomic instability is the most common enabling characteristic of cancer. Aging can be characterized by progressive functional decline, gradual deterioration of physiological function, decrease in fertility and viability and increase in vulnerability. Also aging is believed to be enabled, amongst others, by genomic instability. DNA repair pathways and cell cycle control processes are therefore vital to organisms, since these processes counteract or prevent genomic instability, and are thought to underlie, when affected, aging and age-related diseases like cancer.

To unravel the functions, mechanisms and pathways involved in the onset of aging and age-related diseases we have investigated several mouse models deficient in either DNA repair capacity (**Chapter 3, 4**), cell cycle control (**Chapter 6**) or both (**Chapter 5**), and compared this to a wild type situation (**Chapter 2**).

The use of mouse models enabled us to investigate cancer and aging in a controlled environment, minimizing possible confounding factors. Additionally, the mouse models can be useful to identify carcinogens that can be harmful to the society and the environment (**Chapter 4**).

This thesis emphasizes the importance of functional DNA repair by investigating the consequences of deficient DNA repair. To assess the 'abnormal', one should first try to investigate or establish the 'normal' situation. We therefore investigated wild type C57BL/6J mice together with two knockout mouse models (also in C57BL/6J genetic background) that are deficient for the Nucleotide Excision Repair (NER) pathway. NER is a very important, versatile and highly conserved DNA repair pathway in mammals, eukaryotes and prokaryotes. **Chapter 2** describes the processes that are apparent over time during the entire aging process using cross sectional time points during the lifespan of the C57BL/6J wild type mice. Here, we show chronological and biological (individual) aging is hard to capture by just a few parameters and both the chronological as well as the biological aspect are of importance. However integrating the two enabled us to verify certain aging markers and propose novel biomarkers related to chronological and biological aging. This study also pointed out that aging is an inevitable but stochastic process that results in many different outcomes and that even in the same animal tissues age differently. Overall in spleen and kidney for example, immune responses appear to be regulated mostly during aging. In liver, energy homeostasis demonstrated to be one of the most regulated processes.

Processes that are believed to facilitate cancer and other age-related characteristics were hard to capture in this setting. However, if specific genes in DNA repair pathways are knocked out, the effect of DNA damage on aging and cancer can be investigated. First, we have characterized the *Xpa* and *Xpc*-deficient mouse models in a comprehensive survival study in **Chapter 3** and demonstrated that deficiencies in NER resulted in an increased mutational load and cancer incidence, together with a significant decrease in survival for *Xpc* mice only. The XPA and XPC proteins belong to the same repair pathway and both have a cancer prone phenotype, but still though we found a divergent outcome. *Xpc* mice showed a broader tumor spectrum than *Xpa* mice. Besides the overlapping increase in liver

tumor incidence, lung tumors were significantly elevated in *Xpc* only. A strong elevated lung tumor incidence was also found in *Xpc* mice with a mixed genetic background (C57BL/6-129) [1].

XPA is functional in both routes of NER, Transcription Coupled Repair (TC-NER) and Global Genome Repair (GG-NER) while XPC only functions in GG-NER. The divergent tumor phenotype of the two mouse models directed us to the possibility that XPC could be functional outside NER as well. Since the incidence of lung tumors was elevated and the mice were only exposed to control feed, water and air, oxidative DNA damage could be the reason for the increased sensitivity exhibited by *Xpc* mice. In **Chapter 3** and **Chapter 4** we directly demonstrated, for the first time *in vivo*, that this is indeed the case. XPC is involved in the prevention and/or removal of oxidative DNA damage. When functional XPC is absent, mutations accumulate significantly upon oxidative stress, which is not the case when XPA is not functional or in wild type mice and cells. Also the anti-oxidant response is decreased and the cell cycle progression appears increased in the *Xpc* mouse model, compared to wild type and *Xpa* mice. These processes would facilitate fixation of oxidative DNA damage into mutations. Recent (mostly *in vitro*) data from other studies support the hypothesis that XPC is functional outside NER and is possibly involved in base excision repair, redox homeostasis or cell cycle control. It has previously been suggested that mutations in internal tumors of XP-C patients could be the result of unrepaired lesions caused by oxidative damage. This is now supported by our *in vivo* data. XP-C patients and carriers of SNPs in this gene could benefit from this knowledge, since they might be more sensitive to other sources of DNA damage besides UV and other bulky adduct inducing agents. These novel findings lead to new understandings of the function of XPC.

The broader tumor spectrum and possible additional functions of XPC could be a useful asset in the search of suitable alternatives for carcinogenicity testing. Currently, the 2-year bioassay in mice and rats is considered as the golden standard to predict carcinogenic features of chemicals. This assay is however extremely time-consuming, expensive and requires many animals for testing.

Our previous studies showed that the *Xpa*p53* model is able to predict carcinogenic potential of chemical compounds with high accuracy and is even able to identify non-genotoxic carcinogens [2;3]. These predictions were accomplished upon 39 weeks (short-term) of exposure and more importantly with a strong decrease in the number of animals needed. As a next step we tested the *Xpc*p53* mouse model in *in vivo* carcinogenicity exposure studies. In **Chapter 5** we showed that the *Xpc*p53* mouse model is an attractive candidate to use in short-term carcinogenicity testing. The model conserves all the beneficial aspects of the *Xpa*p53* mouse model (prediction of both genotoxic and non-genotoxic carcinogens after short-term exposure), but additionally the response to toxicity is similar to the wild type situation. *Xpa*p53* mice are more sensitive towards genotoxicity compared to wild type mice. Consequently the use of lower dosages in *Xpa*p53* mice, compared to wild type, for carcinogenicity testing is required, which is a complicating factor in risk assessment.

The outcome of the short-term carcinogenic exposure to 2-AAF in both *Xpa*p53* as well as *Xpc*p53* mice resulted in a severe increase in bladder cancer incidence. In **Chapter 6** we further characterized the 2-AAF induced bladder tumors from *Xpa*p53* mice. We found an unusual mutation spectrum in the wild type p53 allele, which appeared to be the cause for the increased incidence of the bladder cancers, as described in **Chapter 5**. Besides confirming the very high percentage of *Trp53* mutations in the set of urinary bladder tumors of these mice, we made the surprising discovery that a selection for a functional N-terminal truncated isoform of p53 occurred after 2-AAF exposure. Hereby, we showed

dN-p53 isoform formation does not only occur by alternative splicing events [4], but can also be driven by genotoxic exposure. In the presence of full length p53 the isoform could either have a dominant negative effect, inhibiting both p53 transcriptional activity and p53-mediated apoptosis [5;6]. However, the induction of this p53 isoform could on the other hand possibly have a protective effect in a normal cell where both functional alleles of *Trp53* are present: if one *Trp53* allele is damaged by genotoxic exposure, translocation for degradation of p53 by MDM2 could possibly slowed down, since MDM2 cannot bind to an N-terminal region anymore. The formation of a dN-p53 isoform could therefore putatively result in a prolonged cell cycle arrest or apoptotic response. Previous studies showed an enhanced p53 transcriptional activation [7]. Also, a recent finding in human ovarian tumors indicated that high levels of dN-p53 indicated a favorable role of dN-p53 in patients with mucinous ovarian cancer [8].

Future Prospects

The work of this thesis presents novel insights in DNA repair and cell cycle control mechanisms. But, as is usually the case with research, besides solving a few questions, even more are raised by the obtained results. It is clear that NER is an important asset to counteract aging and age-related diseases like cancer, but deficiencies of different proteins in the NER pathway can result into a divergent pathological outcome (also indicated in Table 2 of **Chapter 1**). These findings also broaden the scope of DNA repair and show that pathways do not solely act on their own, but interact and complement each other even more than previously was assumed. These results urge scientists to look further and explore these mechanisms even more closely. New technologies and research areas of e.g. microRNA and post-translational regulation are an interesting addition to the presented research in this thesis. Methylation, acetylation, ubiquitination, sumoylation and phosphorylation together with regulation by microRNAs are most likely important factors in the regulation of DNA repair and cell cycle control. The available data on these processes is only the tip of the iceberg and should be investigated more thoroughly.

Besides a fundamental approach, these results provide a good starting point for more applied science. The discovery of possible novel XPC functions could be beneficial to XP-C patients and carriers of certain XPC variant alleles, which might be more sensitive towards oxidative stress. Intervention treatments using antioxidants or compounds stimulating antioxidant response are putative beneficial applications. Furthermore, the functions of the several p53 isoforms (and also their closely related family members, isoforms of p63 and p73) are yet to be fully revealed and could possibly be used in cancer therapy if clinical relevance and effects on prognosis is further confirmed.

Additionally, the double transgenic mouse models *Xpa*p53* and *Xpc*p53* proved to be promising alternatives in carcinogenicity testing and can already be directly applied. The *Xpa*p53* mouse model has been adopted by the International Conference on Harmonization Expert Working Group on Safety in combination with the traditional 2-year cancer bioassay in rats for the evaluation of the carcinogenic potential of pharmaceuticals. Under the European REACH policy this model is recommended as a good alternative to the 2-year bioassay. Further research could do the same for the *Xpc*p53* mouse model. Moreover, implementation of these alternatives should be recognized by industries and where necessary encouraged by legislative and governmental bodies. Implementation could mean a strong decrease in the use of animals for carcinogenicity testing, would speed up the

process of identifying harmful carcinogens and be economically beneficial. This would especially hold true when transgenic rat models could also be used as an alternative for the 2-year rat bioassay. With the current available technologies it should be possible to replace the current testing strategy with a more suitable strategy in the near future.

Reference List

[1] M.C.Hollander, R.T.Philburn, A.D.Patterson, S.Velasco-Miguel, E.C.Friedberg, R.I.Linnoila, A.J.Fornace, Jr. Deletion of XPC leads to lung tumors in mice and is associated with early events in human lung carcinogenesis, Proc.Natl.Acad.Sci.U.S.A, 102, (2005) 13200-13205.

[2] C.F.van Kreijl, P.A.McAnulty, R.B.Beems, A.Vynckier, H.van Steeg, R.Fransson-Steen, C.L.Alden, R.Forster, J.W.van der Laan, J.Vandenberghe. Xpa and Xpa/p53+/- knockout mice: overview of available data, Toxicol.Pathol., 29 Suppl, (2001) 117-127.

[3] H.van Steeg, H.Klein, R.B.Beems, C.F.van Kreijl. Use of DNA repair-deficient XPA transgenic mice in short-term carcinogenicity testing, Toxicol.Pathol., 26, (1998) 742-749.

[4] J.C.Bourdon, K.Fernandes, F.Murray-Zmijewski, G.Liu, A.Diot, D.P.Xirodimas, M.K.Saville, D.P.Lane. p53 isoforms can regulate p53 transcriptional activity, Genes Dev., 19, (2005) 2122-2137.

[5] S.Courtois, G.Verhaegh, S.North, M.G.Luciani, P.Lassus, U.Hibner, M.Oren, P.Hainaut. DeltaN-p53, a natural isoform of p53 lacking the first transactivation domain, counteracts growth suppression by wild-type p53, Oncogene, 21, (2002) 6722-6728.

[6] A.Ghosh, D.Stewart, G.Matlashewski. Regulation of human p53 activity and cell localization by alternative splicing, Mol.Cell Biol., 24, (2004) 7987-7997.

[7] R.U.Janicke, V.Graupner, W.Budach, F.Essmann. The do's and don'ts of p53 isoforms, Biol.Chem., 390, (2009) 951-963.

[8] G.Hofstetter, A.Berger, R.Berger, A.Zoric, E.I.Braicu, D.Reimer, H.Fiegl, C.Marth, A.G.Zeimet, H.Ulmer, U.Moll, R.Zeillinger, N.Concin. The N-terminally truncated p53 isoform Delta40p53 influences prognosis in mucinous ovarian cancer, Int.J.Gynecol.Cancer, 22, (2012) 372-379.