Chapter 7

Summary and concluding remarks

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It is well known that the tumor suppressor protein p53 plays an important role in protecting cells from becoming mutated and/or transformed into cancerous cells. To accomplish this, the p53 protein fulfills a role in directing cells into, amongst others, cell cycle arrest or apoptosis, both to avoid damaged cells to accumulate and tumors to arise. For efficient initiation of these cellular responses, latent p53 needs to be activated through several post-translational modification events. Phosphorylation of p53 is one of these post-translational regulatory events, which has been studied *in vitro* using transfection assays and *in vivo* using p53 mutant mouse models as reviewed in **Chapter 1**. It became clear that in general and depending on the cellular context, different types of DNA damage induce partly different, but overlapping p53 modifications. From literature, however, it is far from clear how these specific p53 modifications result in certain p53 responses, and how the absence of such modifications affect proper p53 functioning.

The aim of the studies described in this thesis was to gain more knowledge on the role of p53.S389 phosphorylation in a variety of cellular responses, tumor suppression and its possible relationship with DNA repair, more specifically with nucleotide excision repair (NER). For this, several *in vitro* and *in vivo* experiments have been performed using p53.S389A mutant and NER-deficient mice and cells derived from them. *The role of p53.S389 phosphorylation in DNA damage response pathways and tumorigenesis*, being the title of this thesis, was extensively studied. In this final chapter the results are summarized, discussed and directions for future research are indicated.

Is phosphorylation of p53.S389 necessary for p53 for basal maintenance of genome integrity?

Upon exposure to chemical and physical compounds several kinases were found to phosphorylate p53 at serine 389 *in vitro.* Furthermore, several studies indicated that p53.S389 phosphorylation plays a role in p53 tetramer formation, DNA-binding, transcription regulation or growth suppression [1-5]. However, many of these studies are inconclusive, probably because different cell lines were used or unequal p53 protein levels were obtained.

To analyze whether serine 389 plays a role in p53-dependent tumor suppression, mice carrying the p53.S389A mutation in a homozygous fashion were followed as they aged (>2 years). Interestingly, these mutant mice are viable, were born at Mendelian ratios and did not show any obvious abnormalities. It appeared that in the absence of p53.S389 phosphorylation no enhanced spontaneous tumor development occurred as compared to wild-type mice. The tumor spectrum was also identical to that observed in wild-type mice. One has to conclude that p53.S389 phosphorylation is not required for suppression of a spontaneous tumor phenotype, which is in contrast to p53 knock-out mice. In the experiments presented in this thesis, clear levels of phosphorylation of p53.S389 could only be detected after exposure to DNA damaging agents (**Chapter 2**, **4** and **6**). Still, and perhaps in apparent contradiction to these findings, we showed in **Chapters 3** and **5** that basal gene-expression profiles (i.e., without exposure to DNA damaging agents) were changed compared to wild-type in mouse embryonic fibroblasts (MEFs) and in p53.S389A urinary bladders. Apparently, cells and tissues adapt to the new situation (i.e., the p53.S389A mutation), by fine tuning their transcriptional repertoire. This change does, however, not lead to an obvious, detectable, *in vivo* phenotype, at least not in the experimental conditions used by us.

Upon DNA damage we hypothesized, however, that these altered, less ideal, gene-expression levels might lead to a different cellular response in p53.S389A MEFs as well as in intact tissues.

Indeed, we were able to show (**Chapter 3** and **5**) that blocked p53.S389 phosphorylation leads to delayed and/or less efficient responses to DNA damage (see also next paragraphs).
All these possibilities are likely to have only minor effects on spontaneous *in vivo* phenotypes, indicating once more that the p53.S389A mutation is directly linked to external stressors like DNA damage.

Is phosphorylation of p53.S389 necessary for p53 to exert its cellular function?
After DNA damage, p53 is able to induce, amongst others, either a cell cycle arrest or apoptosis. By generating a p53.S389A mouse model, we were able to analyze whether phosphorylation plays a role in these cellular processes.
After exposure to UV radiation, wild-type MEFs rapidly underwent apoptosis, whereas homozygous mutant p53.S389A MEFs showed a reduced apoptotic response (**Chapters 2** and **6**). After gamma-irradiation, however, p53.S389A MEFs underwent a G1 cell cycle arrest as efficiently as wild-type cells (**Chapter 2**). Thus, depending on the type of DNA damage the p53.S389A mutation seems to influence the cellular response.
Next, expression of activated oncogenes in MEFs results in the induction of p53-dependent apoptosis [6]. Upon retroviral infection with adenovirus oncogene E1A it appeared that p53.S389A MEFs have an equal apoptotic response after doxorubicin treatment compared to wild-type cells, whereas $p53^{-/-}$ cells were resistant. This indicates that p53.S389 phosphorylation is not required for oncogene-mediated cell death in cell culture (**Chapter 2**). Upon gamma-irradiation thymocytes undergo p53-dependent apoptosis both *in vivo* and *in vitro* [7]. After exposing p53.S389A mice to gamma-irradiation, we isolated the thymocytes and measured apoptosis. The apoptotic response in the mutant cells again appeared to be comparable to wild-type cells, whereas $p53^{-/-}$ thymocytes were resistant to apoptosis (**Chapter 2**). Also *in-vitro* exposure of p53.S389A-derived thymocytes to doxorubicin did not result in resistance or reduced capacity to initiate apoptosis. In general, we concluded that the p53.S389A mutation does not have a detectable effect on the responses counteracting DNA strand breaks, but it does affect UV-driven cellular responses.

Is phosphorylation of p53.S389 necessary to suppress carcinogen-induced tumorigenesis?
So far, it seems that the p53.S389A mutation only has an effect on cellular responses upon the introduction of certain types of DNA damage. To analyze the potential adverse effect of this feature on tumor suppression, we conducted several chronic carcinogenicity studies with the mutant mouse model. As p53.S389 phosphorylation seems to be somehow linked to UV-induced DNA damage we started to perform a tumorigenesis study using UV as the challenging agent (**Chapter 2**). The Minimal Erythema Dose (MED) seemed to be identical for p53.S389A mice compared to that of wild-type and $p53^{+/-}$ mice. Apparently, the p53.S389A mutation does not result in an altered acute sensitivity of the skin to UV radiation. We, therefore, chronically exposed all mice with different genotypes to the same daily UV dose. Interestingly, however, p53.S389A mice developed more skin tumors with reduced latency times compared to their wild-type littermates. Still though, the tumor types they developed were identical in both genotypes, i.e., papillomas and squamous cell carcinomas, indicating that the absence of p53.S389 phosphorylation does not influence the progression status of developing skin tumors, a phenomenon observed in p53 knock out mice [8;9]. In total it appeared that the UV-induced tumor responses in p53.S389A mice were less obvious than those found in $p53^{+/-}$ mice. This indicates that p53 is still activated and partly functional in p53.S389A homozygous mutant skin cells. Short exposures (2 days)

to UV *in vivo* showed a reduced number of p53 protein positive cells in the skin of p53.S389A mutant mice compared to wild-type mice, but differences between the two genotypes were small again. It is reasonable to assume that for optimal activation of p53 through phosphorylation more sites, i.e., not only the p53.S389 site, need to be modified. Inactivation of one phosphorylation site only has a limited effect on p53 activity, even under stressed conditions. Probably multiple sites need to be inactivated to completely inactivate the protein. Indeed, it was already shown *in vitro* that cooperative phosphorylation at multiple sites is required to completely activate p53 after UV radiation [10].
We tried to generate a p53.S23A mutation in combination with the p53.S389A mutant (collaboration with G. Lozano, results not shown) in mice by homologous recombination experiments, but unfortunately, our attempts of generating such multiple mutants failed.
We, furthermore, checked the p53.S18 (p53.S15 in human) phosphorylation status in UV-exposed p53.S389A mutant MEFs and compared this with the wild-type situation. No differences in p53.S18 phosphorylation levels were observed between the genotypes (data not shown), indicating that in MEFs phosphorylation of p53.S18 is independent of the p53.S389 phosphorylation status after exposure to UV.
A high frequency of missense mutations was found in the *p53* gene of UV-induced skin tumors isolated from p53.S389A mutant mice (**Chapter 4**). These mutations included the (skin) hotspot mutation at codon 270 [11]. Interestingly, another *p53* hotspot mutation at codon 210 in UV-induced skin tumors of p53.S389A mice was found. This mutation was previously assigned as being specific for a defect in NER [12;13]. This indicates that phosphorylation of p53.S389 and NER activity might be interrelated either directly or indirectly (see also next paragraphs).
As a control to the above we exposed mice to gamma radiation (**Chapter 4**), as it is known that under these conditions phosphorylation of p53.S389 does not occur. As expected no increased tumor development in response to gamma radiation of the p53.S389A mice was observed. This *in vivo* experiment confirmed our hypothesis that lack of p53.S389 phosphorylation does not have a negative impact on the role of p53 as a tumor suppressor after exposure to compounds that induce DNA (double) strand breaks.

To further confirm our hypothesis that phosphorylation of p53.S389 is related to compounds that lead to DNA damage that is substrate to NER, we performed an additional carcinogenicity experiment, in which mice were exposed to another compound, i.e., 2-AAF, that leads to DNA damage that is substrate to NER (**Chapter 4**). Interestingly, the p53.S389A mice showed, compared to wild-type mice, an increased incidence of bladder tumors and pre-neoplastic lesions (atypia) after exposure to 2-AAF. This finding further demonstrated the importance of functional phosphorylation of p53.S389 for the response to certain types of DNA damage.
Previously, it was shown [14] that in complete absence of p53 a clear increase of *lac*Z gene mutation frequencies upon 2-AAF exposure was observed in bladder tissue compared to wild-type mice. We investigated whether differences in numbers of mutated cells could be found between wild-type and p53.S389A bladders. After 12 weeks of 2-AAF exposure, a significant increase in *lac*Z mutant frequencies was observed in urinary bladders of both wild-type and p53.S389A mice. However, no significant difference in gene mutation levels between the genotypes was observed (data not shown). Clearly, complete lack of p53.S389 phosphorylation is not sufficient for divergent *lac*Z mutation induction compared to wild-type, at least not in the experimental design tested here.

We identified that p53.S389 phosphorylation is important in apoptotic responses (**Chapters 2, 3** and **5**). To further substantiate this we determined apoptotic responses in 2-AAF-treated urinary bladders of wild-type and p53.S389A mice. Since under these conditions no detectable apoptotic responses could be observed using active caspase-3 staining, we crossed p53.S389A and wild-type mice with DNA repair deficient $Xpa^{-/-}$ mice, which have increased levels of apoptosis upon exposure to genotoxicants [14]. A slight, but not significant, reduction in the apoptotic response of $Xpa^{-/-}$/p53.S389A bladders was found as compared to $Xpa^{-/-}$ bladders (data not shown).
Finally, induction of cell proliferation in urinary bladders was also measured after 1 or 2 weeks upon 2-AAF treatment [14]. No significant difference between wild-type and p53.S389A urinary bladders was observed, although p53-deficient mice showed an increased sensitivity to the induction of cell proliferation in the urinary bladder after exposure to chemical agents [15]. Probably a complete absence of p53 is needed to give such an increase in cell proliferation due to absence of induction of some target genes.
To get more mechanistic insight in these cellular responses we performed microarray analyses (**Chapter 5**) to determine early transcriptional changes that might underlie the cancer-prone phenotype of the p53.S389A mutant mice (see section 'Does the p53.S389A mutation affect transcriptional activation of (target) genes').

Is phosphorylation of p53.S389 related to NER activity?
It is still puzzling how it is determined which cellular responses are induced by p53 upon exposure to certain types of DNA damage. For example, MEFs exposed to UV undergo apoptosis, whereas exposure to gamma radiation does not induce such a response, but rather initiates responses to prevent cells to enter the S-phase of the cell cycle. It is tempting to speculate that specific DNA damages result in specific p53 modifications (for example a chain of phosphorylation and acetylation events), which in turn directs p53 into a downstream response route. In **Chapter 6** we showed that phosphorylation of p53.S389 does not have a role in directing MEFs into either apoptosis or cell cycle arrest events in MEFs. Cellular responses appeared to be more dependent on the specific compound rather than on p53.S389 phosphorylation in particular, indicating that other modifications or events direct the p53 response. Recently, there are indeed some events described in relation to p53 activity, which are able to favor one response over another [16]. For example, phosphorylation of serine 18 and 23 are necessary for apoptosis and tumor suppression, but not for cell cycle arrest and senescence. Furthermore, in human cells phosphorylation of serine 46 is important in favoring apoptotic responses. The cell cycle arrest response is for example favored when p53 cooperates with the transcription factor Miz. This combination is thought to activate *p21*, a well known p53 target gene involved in cell cycle arrest responses. Clearly however, serine 389 phosphorylation of p53 is not involved in these selective actions to initiate specific cellular responses.
Next, we investigated whether specifically DNA damages that are substrate to NER resulted in p53.S389 phosphorylation, or whether phosphorylation of p53.S389 is induced by a group of compounds not physically or functionally related. No conclusive correlation between DNA damages that are substrate to NER and phosphorylation of p53.S389 could be found in our studies. Exposure to compounds that lead to DNA damage that is substrate to NER, but also compounds which induced DNA damage that is substrate to a different repair system, led to p53.S389 phosphorylation (**Chapter 6**). We cannot exclude, however, that certain compounds

have broader lesion spectra (including those related to NER) than we currently assume. Still though, DNA damages that are substrate to NER, especially those induced by UV, did result in relatively the highest levels of p53.S389 phosphorylation compared to for example BER-related compounds.

Therefore, we assumed that it is still possible that p53 modifications can influence NER activity. Direct evidence for such an involvement came from the observation that when p53 is absent in human cells a reduction in UV-induced DNA damage repair was measured [17;18]. Looking in more detail at the contribution of p53 in NER it was shown that p53 is required for efficient GG-NER, whereas TC-NER did not require p53 [19;20]. Although we did not find a conclusive correlation between compounds that lead to DNA damage that is substrate to NER and phosphorylation of p53.S389, we still found evidence that p53.S389A mutant mice and cells derived from them are more sensitive to compounds that lead to DNA damage that is substrate to NER than they are to other compounds [21-23]. This observation points towards the assumption that cells lacking p53.S389 phosphorylation have less efficient NER activity.

In a first attempt to find a correlation between p53.S389 phosphorylation and NER activity we measured unscheduled DNA synthesis (UDS) after UV treatment (data not shown). No differences, however, were measurable in UDS activity between wild-type, p53.S389A or $p53^{-/-}$ MEFs. Although p53 was found to be required in human cells for efficient GG-NER after exposure to UV [19], our results in mice indicated that phosphorylation of p53.S389 is not needed for GG-NER in mice. However, these results can be explained by the fact that rodent cells lack functional p53 responsive elements needed to transactivate the *Xpc* and *Xpe* genes, which might be the reason that rodents have inefficient GG-NER activity [24-26]. Thus, types of DNA damage normally repaired through XPC and XPE in humans are repaired with other p53-independent kinetics in rodent cells. This might well be the reason that UDS-analysis did not display any difference between wild-type and p53-deficient MEFs, and as a consequence, phosphorylation of p53.S389 is not needed for optimal GG-NER functioning in mouse cells.

Based on these results we hypothesized that persistent DNA damage in actively transcribed DNA, resulting in a blockage of transcription, might well be the initiating stimulus of phosphorylation of p53.S389. A similar correlation between blocked transcription elongation and phosphorylation of p53.S15 was recently found by others [27].

Model linking stalled RNA polymerases (TC-NER) with p53.S389 phosphorylation

Combining all findings so far, we can postulate a model for the role of p53.S389 phosphorylation in the activation of NER (Figure 1). This model shows the two pathways of NER, TC-NER and GG-NER, of which TC-NER is thought to be related to apoptosis induction, whereas GG-NER is thought to be related to repair mechanisms. It was previously concluded [27] that inhibition of transcription is sufficient for the accumulation of p53 in the nucleus regardless whether p53.S15 is phosphorylated or not. More importantly, it was shown [27] that blockage of transcription elongation triggers a distinct signaling pathway leading to p53 modifications at serine15.

The triggering event for p53.S389 phosphorylation still remains unclear, but since p53.S389 phosphorylation most abundantly occurs after exposure to compounds that lead to DNA damage that is substrate to NER, we hypothesized that stalled RNA polymerases by DNA damages that are substrate to NER present in actively transcribed DNA results in p53 phosphorylation at both serine 389 (this thesis) as well as serine 15 [27].

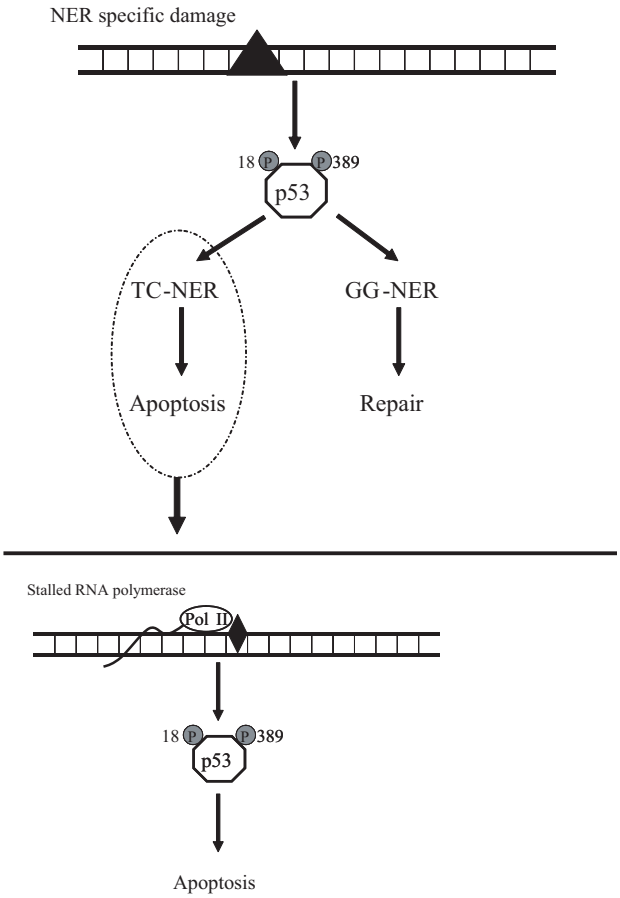

Figure 1 - Model for the role of p53.S389 phosphorylation in relation to NER
Due to NER-specific DNA damaging compounds (upper part) or through inhibition of RNA synthesis by a stalled polymerase (lower part), p53 is activated by post-translational modifications at serine 18 and serine 389. This directs the NER activity into TC-NER finally leading to apoptosis (upper part) or immediately into apoptosis (lower part).

Our experiments with NER-deficient cells clearly provide evidence for this hypothesis, since cells lacking TC-NER ($Xpa^{-/-}$ and $Csb^{-/-}$) induced high levels of p53.S389 phosphorylation even at low UV doses, whereas cells only lacking GG-NER ($Xpc^{-/-}$) are indistinguishable from wild-type cells (this thesis, **Chapter 6**). Our hypothesis was further substantiated since doxorubicin, which is amongst others a RNA polymerase inhibitor, also gives rise to p53.S389 phosphorylation (**Chapter 6**). However, as doxorubicin is also an inhibitor of DNA polymerases, we cannot exclude the possibility that blocked DNA replication also leads to p53.S389 phosphorylation [28-30].
Finally, it is clear that actively transcribed DNA strands need to be free of any DNA damage, to allow proper and efficient transcription. Therefore, one can envision that cells with severe DNA damage on transcribed strands will rapidly undergo apoptosis, a phenomenon recently described by others [31;32]. This hypothesis is also underscored by our previous finding that lack of p53.S389 phosphorylation in MEFs after exposure to UV resulted in decreased apoptotic responses (**Chapter 2**).

Does the p53.S389A mutation affect transcriptional activation of (target) genes?
New innovative technologies, like microarray analyses, were used to analyze gene expression levels

on a genome wide scale to study p53 functionality; since it is well-known that p53 functions as a transcription factor. To investigate specific effects of the p53.S389A mutation on transcriptional responses, two different microarray analyses were performed. In **Chapter 3** the role of p53.S389 phosphorylation on transcriptional changes upon UV exposure in MEFs was described. **Chapter 5** described the identification of early transcriptional changes underlying the 2-AAF-induced cancer-prone phenotype of p53.S389A mice. Interestingly, in both *in vitro* as well as *in vivo* analyses we found differences in basal gene expression levels between p53.S389A and wild-type. The number of genes with adjusted basal gene-expression level prior to exposure seems rather high in MEFs (**Chapter 3)**. Since this was an *in vitro* experiment using cells in culture an explanation for this could be the presence of relatively high levels of so-called 'spontaneous' DNA damages, like reactive oxygen species (ROS) or depurination [reviewed in [33]], since, related to this, cells were exposed to 20% O_2 and 6% CO_2 inducing (genotoxic) stress on the cells.

We, furthermore, hypothesized that the intrinsic cellular transcription machinery might be re-adjusted as a whole network to adapt to the effect of the introduced p53.S389A mutation, thereby resulting in differences in basal gene expression activity.

In **Chapter 1** we already described that p53 can selectively repress genes to affect a cellular response through direct binding to a specific consensus DNA binding element [34]. It was, furthermore, shown that p53-dependent repression in response to DNA-damage was also possible without binding to this consensus sequence. P53 may, therefore, act directly or indirectly through a DNA binding protein complex at promoters of repressed genes. Interestingly, the microarray experiment described in **Chapter 3** showed us that p53-dependent repression of gene-expression can be affected by just a minor p53 modification (i.e., p53.S389A) in a similar fashion as complete absence of p53 would do. In contrast, for p53-dependent induction of gene-expression the effect of p53 absence cannot as easily be mimicked, probably due to redundancy in activation mechanisms.

Overall, gene expression levels after exposure to the DNA damaging agents that are substrate to NER like UV (*in vitro*) or 2-AAF (*in vivo*) were altered in p53.S389A mutant cells, since they were found either reduced or delayed, respectively, compared to wild-type (**Chapters 3** and **5**). In both settings, several well-known p53 target genes involved in apoptosis and cell cycle arrest were found adversely affected by the presence of the p53.S389A mutation. In both experiments, differences between wild-type and p53.S389A expression profiles were usually minor, in contrast to differences found with p53 knock out cells. The question, therefore, arises whether these small changes in expression levels of target genes can account for the increased tumor responses we observed (**Chapters 2** and **4**). It is well known tumors develop as a result of the accumulation of various molecular changes, rather than single genes or pathways becoming affected. Subtle effects in a broad group of genes and pathways especially when found combined, as observed in our studies, might direct cells into (pre)malignant lesions, ultimately resulting in tumor outgrowth.

In conclusion, phosphorylation of p53.S389 appears to play a role in fine-tuning the expression of a variety of important genes instead of transcriptional activation of a few genes with key regulatory functions.

Final Remarks

Our strategy to develop a physiologically relevant mouse model appears to be valuable for analyzing the importance of specific phosphorylation sites on p53 functioning. Using these mice, phosphorylation of p53.S389 was identified to be involved in some, but certainly not all p53 functions.

In this thesis we showed the contribution of p53.S389 phosphorylation on p53 functioning in a variety of processes (data are summarized in Table I). After DNA damage, p53 protein levels increase due to several post-translational activation processes. Phosphorylation of p53.S389 seems to be partly required for optimal induction of these p53 protein levels. Next, target genes are either induced or repressed, and phosphorylation of p53.S389 seems essential for an optimal p53-related transcriptional response both endogenously (especially repressed genes) as well as after the induction of DNA damage. Than as a read-out system for the activation of different genes, several cellular responses (apoptosis, cell cycle arrest etc.) can be observed, which again seems partly dependent on p53.S389 phosphorylation. When these processes are adversely affected due to inadequate functioning of p53, like is the case in p53.S389A mutant mice, this might lead to increased risks of developing tumors. Indeed, two chronic carcinogenicity experiments revealed an increased sensitivity of the p53.S389A mutant mice for tumor development upon exposure to DNA damaging agents.

Table I - Phenotype of p53.S389A mice and cells derived from them

		In vitro	
Cells	**Agent**	**Results compared to wild-type**	**Chapter**
MEFs	UV-C	Reduced induction of p53 protein levels	2
		Reduced apoptotic respons	2
		Reduced DNA binding activity	2
		No difference in p53.S15 phosphorylation level	DNS
		No difference in UDS	DNS
		Affected basal (i.e.without exposure) gene expression level	3
		Altered biphasic response	3
	Gamma radiation	No difference in induction p53 protein levels	2
		No difference in G1-arrest	2
	Oncogene E1A	No difference in oncogene-mediated cell death	2
	Various	No compound specificity for phosphorylation of Ser389	6
		No specific regulation in directing cells to a cellular response	6
Thymocytes	Doxorubicine	No difference in apoptotic response	2

DNS = data not shown, UDS = Unscheduled DNA synthesis

The results presented in this thesis provide new information on the role of a specific point mutation in p53 in chemical-induced tumorigenesis. In addition, knowledge about the *in vivo* relationship between DNA damage induction, regulation of p53 activity (in terms of cell cycle control and/or apoptosis), DNA repair (NER) and the development of cancer was obtained. Further studies are needed to unravel the specific functions of p53.S389 phosphorylation in DNA repair, with specific focus on NER. To this end it would be helpful to cross our developed p53.S389A mutant mice with those lacking specific functions related to NER in either GG-NER or TC-NER. As a final point, it will be worthwhile in the future to generate and analyze mice with combinations of mutations in regulatory sites in p53, including the p53.S389A mutation.

The experiments described in this thesis aimed at understanding the role of serine 389 phosphorylation in proper p53 functioning were restricted to mice and cells derived from them. Whether absence or decreased efficiencies of p53.S389 phosphorylation (i.e., serine 392 in human) are also of relevance in humans, and as such account for increased sensitivity for sun-light-induced skin cancers, is an interesting question that needs to be investigated.

In vivo

Tissue	Agent	Results compared to wild-type	Chapter
Whole mice	X	No difference in Mendelian ratios	2
		No obvious abnormalities	2
		No difference in spontaneous tumor development	2
Whole mice	Gamma radiation	No difference in tumor development	4
Thymocytes	Gamma radiation	No difference in apoptotic response	2
Skin	UV-B	Increased tumor development	2
		Slightly induced tumor yield	2
		No difference in tumor type	2
		Reduced amount of p53 protein	2
		p53 mutations found at codon 210 and 270	4
Urinary bladder	2-AAF	Increased tumor development	4
		No difference in *lac* Z mutant frequencies	DNS
		Slight difference in apoptotic response*	DNS
		No difference in cell proliferation	DNS
		Affected basal gene expression level	5
		Delayed expression of apoptotic and cell-cycle control genes	5

* Mice crossed with $Xpa^{-/-}$

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