Delineating the DNA damage response using systems biology approaches

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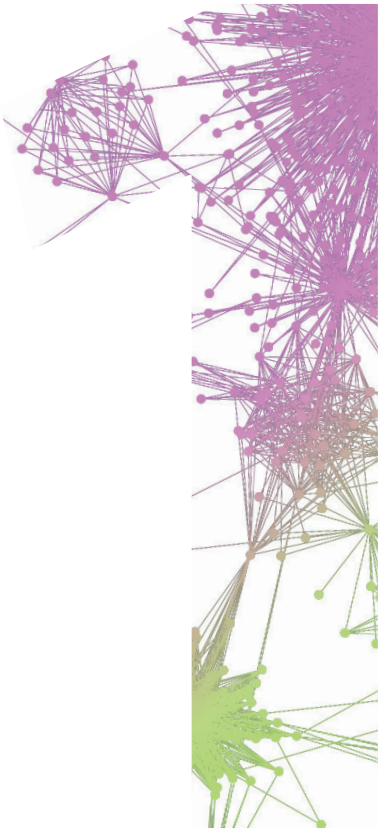
GENERAL INTRODUCTION AND SCOPE OF THIS THESIS



PARTS OF THIS CHAPTER ARE ACCEPTED AS A BOOK CHAPTER IN TOXICOGENOMICS AND ALTERNATIVES TO CURRENT ANIMAL MODELS FOR SAFETY ASSESSMENT

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ABSTRACT

Imperfect repair of damaged DNA caused by exogenous (e.g. UV radiation) or endogenous sources (e.g. metabolic reactive oxygen) may be a driving force in evolution but also contributes to cancer and ageing. For radio- or chemotherapy, accumulation of DNA damage is the major mechanism of action as well as the dose limiting toxicity to normal tissues. Sensing of DNA damage initiates an intricate signaling response, ensuring a cellular outcome, which is adequate to the type, amount and duration of the damage, and is at the same time highly dependent on the cellular background. As described in the chapters of this thesis we used the integration of high-throughput techniques to decipher DNA damage-induced cellular responses in embryonic stem cells and cancer cells.

DNA damage sources and damage sensing

DNA is the only biomolecule that cannot be recycled and instead is being repaired. A plethora of extrinsic and cell-intrinsic factors, including genotoxic substances, UV- and γ–irradiation, as well as endogenous stresses, such as metabolically arising reactive oxygen species (ROS) and replication errors, pose threats to the integrity of our genomes, by inducing various kinds of DNA lesions [1; 2.] Together these insults result in up to one thousand DNA lesions per cell per day [1]. Small DNA lesions, such as base modifications and base mismatches are potentially mutagenic but do not interfere with global DNA functioning. Bigger DNA lesions, such as bulky adducts, double strand breaks (DSBs) or DNA crosslinks interfere with DNA replication and transcription, and enzymes involved in those processes often function as damage sensors [3]. Other damage sensing mechanisms are intimately linked with DNA repair pathways, which are designed to repair specific types of lesions [4].

Recognition of DNA damage elicits a complex signaling cascade, resulting in the arrest of the cell cycle to allow time for repair and avoid the passage of potentially damaged genetic material. Damage beyond repair, can provoke initiation of apoptosis, senescence or differentiation, as a safeguard mechanism to remove damage-carrying cells from the tissue or the lineage [5; 6] (Fig 1). If the successful execution of this DNA damage response (DDR) signaling cascade fails, cellular consequences can be cell death through mitotic catastrophe or necrosis. Survival of damage-carrying cells bears the risk of accumulation of mutations and chromosomal aberration, possibly resulting in neoplastic transformation [5; 7].

The DDR is central to the balance between cancer formation and ageing on the one hand, and for many types of cancer therapy on the other hand. Studying the underlying signaling responses is vital for a better understanding of these processes. It has emerged in recent years that the DDR, rather than being a simple downstream transduction cascade, constitutes a highly complex signaling network, which is dependent on cellular and organismic background, as well as damage

type, intensity and duration (Fig 1). Moreover, the DDR signaling network has to integrate DNA damage-initiated processes, such as repair, with cellular housekeeping pathways, such as ongoing transcription, translation or DNA replication as well as other signaling pathways, which are for example involved in survival signaling and development[5; 8; 9] (Fig 1).

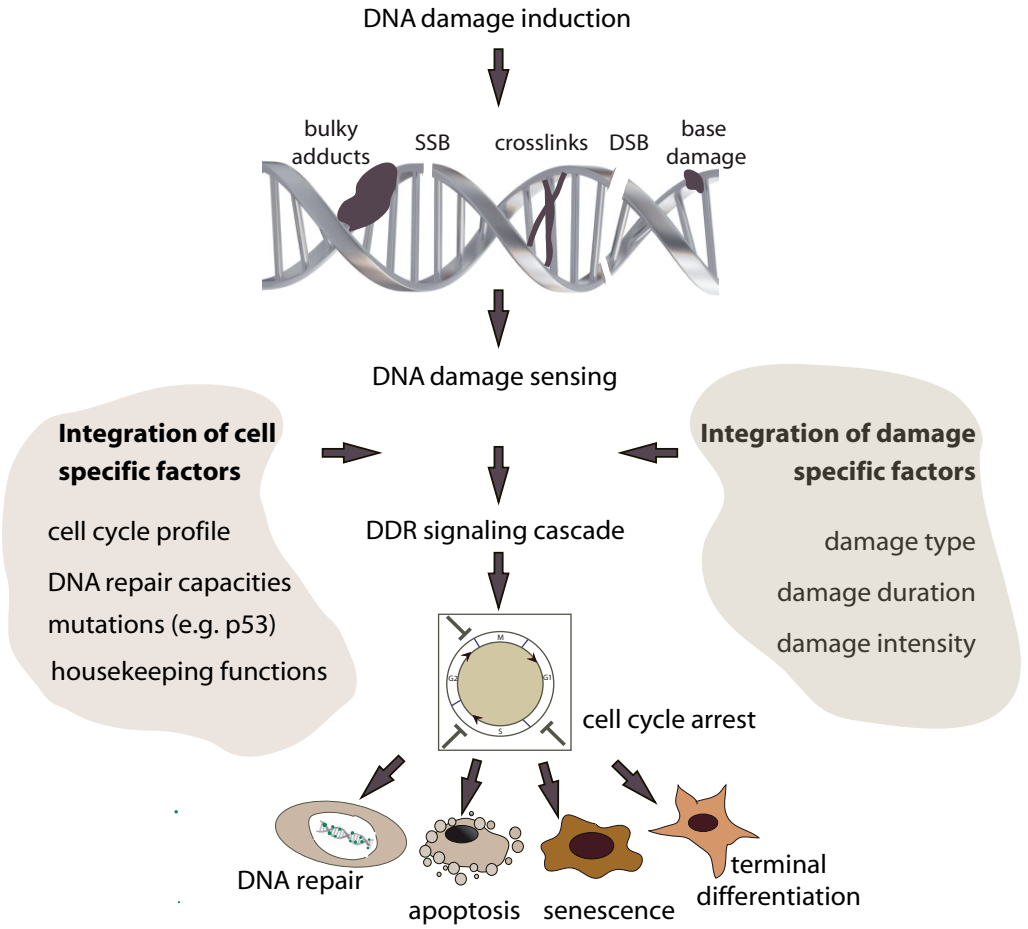

Figure 1. DDR signaling responses. Specific types of DNA damage lead to the induction of distinct DNA lesions, including base damage, bulky DNA-adducts single strand breaks (SSB), double strand breaks (DSB) and intra-strand crosslinks. The presence of such lesions elicits a signaling cascade that is integrated with ongoing cellular activities (e.g. cell cycle, transcription, translation), cell specific factors (e.g. DNA repair capacity), and damage specific factors (e.g. type, duration and intensity of the damage). Altogether, this determines the cellular outcome, which typically starts with a cell cycle arrest and subsequent effective DNA damage repair or, if repair fails, removal of cells from the tissue through senescence or cell death (for example by apoptosis) or terminal differentiation.

Signal transduction in the DDR depends on a phosphorylation cascade

Sensing of DNA lesions turns on a quick signaling reaction, which is crucially dependent on the addition of posttranslational modifications to signaling molecules and chromatin components, comprising phosphorylation, ubiquitination, sumoylation, methylation and ribosylation [10]. Key players in the DNA damage-induced phosphorylation cascades are the PI3-K-related protein kinases ataxia-telangiectasia mutated (ATM), ATM and RAD3 related (ATR) and DNA-dependent protein kinase (DNA-PK) [11; 12].

After DNA damage, ATM and ATR alone lead to phosphorylation of more than 700 proteins, with a total number of 900 phosphorylation events [13]. The substrates of ATM and ATR reflect the whole spectrum of the DDR and are involved in cell cycle arrest, DNA repair and cell survival [11; 12]. Whereas ATR is vital for the survival of replicating mammalian cells, ATM-deficient cells are viable. However, mutations in ATM result in the cancer predisposition genetic disorder ataxia-telangiectasia (AT), which is characterized by cerebellar ataxia, immunodeficiency, genomic instability and cancer predisposition [11].

The signal for the recruitment of ATM is the presence of DSBs, which are recognized by the Mre11, Rad50 and Nbs1 (MRN) complex [2]. The crucial lesion for the recruitment of ATR is Replication protein A (RPA)-coated single strand DNA, which is sensed by ATR interacting protein ATRIP and further requires the Rad9-Rad1-Hus1 (9-1-1) clamp loading complex and the ATR activator Topoisomerase binding protein 1 (TOPBP1) [12]. Single stranded DNA often arises during replication stress, or as a result of resected DSBs. DSBs are a common secondary DNA lesion, resulting for example from single strand breaks, which encounter replication forks. Thus, although initially recruited by different kinds of lesions, ATM and ATR frequently cooperate in the DDR signaling response [2]. In the DSB response ATM is rapidly activated at any stage of the cell cycle, while the activation of ATR occurs more slowly and is mostly limited to S and G2 [12].

ATM and ATR share many common targets and only a few substrates are exclusive for one of the kinases, e.g. the checkpoint kinases Chk2 and Chk1 that diffuse through the cell to further convey the damage signal [14]. Similar to ATM, also Chk2 is dispensable for viability, but mutations cause Li Fraumeni syndrome, a hereditary cancer susceptibility syndrome, also associated with mutations in p53 [11]. Conversely, Chk1, like ATR is crucial for viability, with cells of Chk1 knockout mice displaying a phenotype that resembles mitotic catastrophe [14]. ATM and ATR, as well as Chk1 and Chk2 can activate p53 by leading to the disruption of the regulatory loop that exists between p53 and its negative regulator MDM2 through phosphorylation of both proteins [14; 15; 16]. Despite the undoubtedly crucial role of ATM and ATR as key players in the DDR, recent reports have implicated other kinases as important signal transducers in the genotoxic stress response [17; 18]. For instance, p53-deficient cells have been shown to rely on a p38MAPK/MK2 signaling module for checkpoint activation [19; 20].

The transcription factor p53 serves as a central hub in the cellular stress response

The transcription factor p53, known as the "guardian of the genome" regulates a variety of cellular processes ranging from apoptosis, cell cycle arrest, autophagy, DNA repair and senescence to metabolic processes, angiogenesis, differentiation and immune responses [21; 22; 23; 24; 25]. It can execute these functions both in a transactivation-dependent and -independent manner [25; 26]. Through its action as a transcription factor p53 regulates a great number of target genes, including not only protein coding genes but also miRNAs, such as the proapoptotic miR-34 family [27]. Amongst the protein coding genes the key proapoptotic target of p53 is the BH3-only protein PUMA. However, unlike p53, PUMA-deficiency by itself does not lead to tumor formation in mouse models [28]. Next to this transcriptional effect on apoptosis, p53 can also physically translocate to the mitochondria to directly cause apoptosis via induction of BAK oligomerization and cytochrome c release [29; 30].

Besides apoptosis, p53 also regulates G1 arrest via the upregulation of p21 or GADD45; as well as senescence, which is mediated by induction of plasminogen activator inhibitor, but can also be activated by p21 [25]. Similar to the effect of PUMA-deficiency, lack of p21 is not by itself tumorigenic, indicating that more than one p53 target gene is important for the mediated effect [31]. p53 regulated genes are involved in the induction of autophagy [24] and regulate cellular metabolism for example by acting on mTOR [23]. Moreover, p53 also controls a number of other pathways including DNA repair and antioxidant regulation [32; 33].

The plethora of p53-mediated responses points to a crucial role of p53 as a central hub protein that functions to monitor and integrate a vast amount of information to finally determine the cellular outcome based on the context of cell, tissue and organism; as well as type, duration and amount of stress. Indeed, a lack of p53 or deficiency in p53 function is common for many cancer types, mostly rendering cells resistant to genotoxic stress-induced killing [34]. However, in certain cellular contexts, lack of p53 can also enhance sensitivity, mostly as a consequence of deregulated cell cycle checkpoints and resultant mitotic catastrophe [19; 35; 36.]

p53 is regulated by posttranslational modifications

The stability, localization and transactivation capacity of the tumor suppressor p53 is regulated by a complex network of posttranslational modifications, including phosphorylation, ubiquitination, sumoylation, neddylation, methylation, acetylation, and glycosylation. Over 36 different amino acids of p53 have been shown to be modified, sometimes in a stress-induced manner [37]. Regulation by ubiquitination is prominent: in unstressed conditions p53 levels are held low by MDM2-mediated ubiquitination and subsequent proteasomal degradation. The E3 ubiquitin ligase MDM2, in turn, has the capability of inducing self-ubiquitination, but is targeted for proteasomal degradation by other ubiquitin ligases [38]. Moreover, MDM2 itself is a target gene of p53 providing an autoregulatory feedback loop [39]. The MDM2 gene is amplified in 7% of human cancers and the critical role of MDM2 in regulation of p53 is underscored by the fact that p53

deletion can rescue embryonic lethality in MDM2-deficient mice [40]. Another player in the ubiquitin-mediated regulation of p53 is MDM4 (MDMX in mice), which despite being structurally related to MDM2 has no reported ability to ubiqutinate p53, but acts as a structural interactor for p53 and MDM2 and can both activate and repress p53 [41; 42].

In addition to the MDM2/MDMX module other ubiquitin ligases are involved in regulating p53 stability and localization, such as COP1, Pirh2, ARF-BP1, MSL2 and Parc [43]. Ubiquitin removal by deubiquitinases (DUBs) provides a further mode of p53 regulation. The deubiquitinase HAUSP, also known as USP7 can deubiquitinate p53 as well as MDM2 [44]. The main function of USP7 seems to lie in the regulation of MDM2, since its ablation stabilizes p53 levels [44; 45]. USP10 removes MDM2-mediated ubiquitin chains from p53, thereby preventing p53 nuclear export and degradation. USP10 itself is stabilized after DNA damage and a fraction of this protein localizes to the nucleus, a process regulated by ATM-mediated phosphorylation [46]. USP4 indirectly regulates p53 by deubiquitinating and thereby stabilizing the negative regulator ARF-BP1 [47].

Other posttranslational modifications can lead to p53 stabilization by hindering the addition of ubiquitin, such as phosphorylation of p53 and MDM2 after stress by kinases such as ATM, ATR, DNA-PK as well as the checkpoint kinases [37]. In vivo studies using knock in mice in which single or double phosphorylation sites in p53 are mutated have shown that no single phosphorylation event is exclusively responsible for stabilization of p53 after stress [37]. Recently it has been established that DNA damage-induced stabilization of p53 protein levels occurs as an oscillatory response, pulses depending on activation of p53 by ATM which leads to induction of negative regulators MDM2 and Wip1, subsequently decreasing p53 levels [48; 49]. Finally, posttranslational modifications can change the transactivation features of p53 and affect the interaction with different transcriptional co-activators or repressors. Acetylation of p53 by the histone acetylase CBP/ p300 enhances protein stability, sequence specific DNA binding and interaction with cofactors and further leads to acetylation-dependent chromatin relaxation in p53 target genes [50].

DNA damage-induced cellular outcomes

DNA repair

The variety of DNA repair pathways matches the diversity of different lesions that can be induced in DNA. Generally one can distinguish repair pathways that act on damage affecting only one strand of the DNA including single strand breaks, mismatches and smaller base modifications; and pathways which act on damage affecting both strands, such as DSBs and crosslinks [51].

Repair of small DNA lesions

Pathways dealing with smaller kinds of DNA damage include mismatch repair (MMR), a strand specific repair mechanism that corrects base mismatches occurring during DNA replication but also participates in a variety of other DNA transactions [52], and base excision repair (BER). The BER pathway serves to remove small DNA

lesions such as base alterations, including oxidative modifications, methylations or alkylations, as well as single strand breaks (SSBs) [51; 53]. Lesions can be either mutagenic or cytotoxic/ cytostatic and therefore the failure of different DNA repair pathways will result in different cellular outcomes [1]. Small lesions, which are being repaired by MMR and BER do not interfere with the helix, but might, if not repaired, result in mutations.

Repair of bulky and helix interfering DNA lesions

Helix distorting, bulky DNA lesions (benzopyrene-induced adducts; UV-induced lesions, e.g. thymine dimers and 6-4-photoproducts; bulky lesions caused by DNA cross linking agents) are repaired by the nucleotide excision repair (NER) pathway. NER is sub-classified into two different types that share a common core pathway, but differ in the damage sensing mechanism. Global genome repair (GGR) functions both in the transcribed and the untranscribed strand and does not require the gene in which the damage occurs to be active. The GGR pathway makes use of DNA damage sensor proteins, such as DDB and XPC-Rad23B in order to recognize helix distortions. Conversely, the transcription coupled repair (TCR) pathway relies on the sensing of DNA damage by RNA polymerase II which gets stalled at bulky lesions [54]. Defects in NER can result in cancer susceptibility syndromes such as Xeroderma pigmentosum (XP) and progeria syndromes such as Cockayne syndrome and trichiothiodystrophy [1; 55].

The repair of intrastrand crosslinks is achieved with the aid of the Fanconi Anemia (FA) repair pathway. FA is a rare, autosomal recessive genetic disease, caused by mutations in at least one out of the 14 complementation group genes. Patients show cancer predisposition, neural, developmental and skeletal abnormalities, aplastic anemia and a high sensitivity to crosslinking agents such as mitomycin C or cisplatin [56; 57].

DSBs are the most deleterious, however, also the most rarely occurring DNA lesion, resulting either from blockage of replication forks caused by other types of lesions, as a result of ROS-induced single strand breaks or can be directly induced by ionizing radiation [58]. Two pathways are used for the repair of double strand breaks: 1) homologous recombination (HR) employing a homologous template for strand replacement and thus being restricted to late S and G2-phase of the cell cycle; and 2) the error prone mechanism non-homologous-end joining (NHEJ), which does not require the presence of a homologous template and can therefore function throughout the cell cycle. The decision for one or the other pathway is dependent on the organism, cell type, cell cycle status, the mode of DSB induction and the chromatin structure surrounding the break [58]. In the NHEJ pathway the two ends of a DSB are ligated together. Crucial for this are the DNA-PK catalytic subunit (DNA-PKcs), as well as the Ku70-Ku80 protein heterodimer that forms a complex on both sides of the DNA ends, which interact and bridge the gap [51]. HR is critically dependent on Rad51, which mediates homology search and strand invasion, between the damaged DNA strand and the homologous template, as well as the breast cancer susceptibility genes BRCA1 and BRCA2 [51; 59].

Many factors are common to more than one repair pathway, such as those involved in HR and FA pathways. Furthermore some lesions such as DNA crosslinks require more than one repair mechanism for their removal, indicating a close interconnection between different repair pathways.

In addition to repair, another way cells can cope with DNA damage is the bypass of the damage during DNA replication, a process known as translesion synthesis (TLS). In this pathway a switch from a high fidelity DNA polymerase to the Y-family of DNA polymerases occurs. These polymerases can carry out replication over damaged DNA, but have reduced fidelity on undamaged substrates, making the process of TLS potentially mutagenic. Different types of polymerases are hereby capable of bypassing different kinds of lesions [60].

DNA damage-induced cell death

In the case of failed DNA repair, cells can undergo different forms of cell death including apoptosis and autophagy, as well as necrosis, senescence and mitotic catastrophe [61]. The apoptotic process is characterized by chromatin condensation and cellular shrinking, and concludes in the formation of apoptotic bodies, membrane surrounded cellular particles, which can be phagocytized by neighboring cells. This prevents undesirable immune responses by the release of intracellular factors into the extracellular space, as induced by necrosis which is characterized by a rapid loss of membrane integrity [62]. Apoptosis features two major pathways. The cell extrinsic or death receptor pathway is regulated by extracellular molecules such as FAS-ligand to the death receptor family membrane receptors (e.g. FAS) and shuts on signaling via the Fas-associated death domain protein (FADD). The cell intrinsic or mitochondrial pathway is regulated by intracellular stress signals, acting via the activation of proteins of the Bcl-2 family. This family includes positive regulators of apoptosis such as BAK and BAX and the BH3 only proteins PUMA, NOXA and BAD as well as negative regulators such as Bcl2 or Mcl1, which modulate the release of cytochrome c from the mitochondria and apoptosome formation [62; 63; 64]. Crucial to apoptosis is the activation of the caspase family of cellular proteases, which together with nucleases carry out cellular breakdown by degradation of proteins and nucleid acids. Despite differences in the earlier steps of the signaling cascade both pathways will eventually culminate in the induction of effector caspases caspase-3, -6, and -7 [63].

Misregulation of many proteins involved in induction of apoptosis such as p53 or Bcl2 family members, as well as inhibitor of apoptosis proteins (IAPs) has been implicated in cancer formation and therapy response [65]. DNA damage induces apoptosis in normal and cancer cells, however, the proportion, which cell death by apoptosis really contributes to the success of chemotherapy is not yet clear. Besides apoptosis, other forms of cellular demise are important consequences of DNA damage induction: senescence and mitotic catastrophe (MC). Senescence describes a terminally arrested state in which cells, although not dividing, are still metabolically active and able to affect neighboring cells by secreting factors. In MC abrogation of G1/S or G2/M checkpoints

leads to mitotic entry in presence of DNA damage, which results in mitotic abnormalities and subsequent death [7; 66; 67]. MC might be especially important in cancer cells, which often carry checkpoints defects. Cells with lack in p53 signaling will undergo mitotic catastrophe if the backup checkpoint axis, which is maintained by p38/Mk2, is silenced [19].

DNA damage-induced cell cycle arrest

Different cell cycle checkpoints have evolved that prevent replication of damaged DNA and premature entry or exit from mitosis, and allow time for DNA repair after encountering DNA damage. The main cell cycle checkpoints are the G1/S-checkpoint, the intra S-Checkpoint and the G2/M-checkpoint [68]. The transition through stages of the cell cycle is regulated by the action of cyclin-dependent kinases, which are key targets for modulations induced by different cellular stimuli, including DNA damage. G1 arrest, which is the main DNA damage-induced checkpoint in non-cancerous cells can be activated via an ATM-Chk2-p53-p21-mediated signaling cascade that culminates in silencing cyclinE/Cdk2 kinase [2]. Further, a faster, transcription-independent route for cell cycle regulation exists, via the CDC25 family phosphatases (comprising CDC25A, CDC25B and CDC25C), which can remove the inhibitory phosphorylations from cyclin-dependent kinases (CDKs). While CDC25A is mainly thought to regulate G1/S checkpoint by activating cyclinE (A)/ Cdk2 kinase, CDC25C is acting on cyclinB/Cdk1, thereby mediating the entry into mitosis and regulating the G2/M checkpoint. However, CDC25 phosphatases are not functionally restricted and in many cases have redundant roles [69]. The Checkpoint kinases Chk1 and Chk2 phosphorylate the CDC25 phosphatases, which attenuates CDC25 protein stability through priming for proteasomal degradation and furthermore induces their interaction with 14-3-3, sequestering them from Cdk1. Both mechanisms result in an induction of cell cycle arrest [69; 70].

Next to DNA damage-induced cell cycle arrest, regulation of checkpoint maintenance and checkpoint recovery is important for cellular survival after genotoxic stress. Polo-like-kinase 1 (Plk1), as well as Aurora kinase A and the phosphatase Wip1 are crucial players in checkpoint recovery after G2 arrest. Plk1 phosphorylates and activates negative regulators of p53 and stimulates nuclear translocation of CDC25B/C, leading to a reinitiating of the cell cycle. The phosphatase Wip1 has been shown to be crucial for cell cycle recovery in p53 proficient, but not deficient cells, being itself a target gene of p53 and at the same time capable of regulating p53 on multiple levels. Wip1 assures the low level expression of cell cycle regulators to enable eventual re-entry into the cell cycle [68].

Differentiation in response to DNA damage

The response to DNA damage strongly depends on the cellular context. In addition to the cell cycle profile, also the differentiation status and interactions of a cell and its environment can determine the cellular outcome of genotoxic stress. In non-dividing, terminally differentiated cells such as neurons, DNA repair pathways can be altered or attenuated [5].

Embryonic development as well as adult tissue homeostasis depends on the function of stem cells, which are characterized by their potential for self renewal and pluri- or multipotency. Accumulation of DNA damage in stem cells will not only affect a single cell, but may be passed to the lineage [71]. Adult stem cells have a higher potential to become malignant since they already encompass a number of cancer cell-like features and their comparably long life span makes them more vulnerable to accumulation of mutations. One hypothesis for cancer formation is that adult stem cells are predecessors of tumor initiating or cancer stem cells [72].

Loss of stem cells by DNA damage-induced apoptosis will affect tissue homeostasis and has been linked to progeria syndromes but may also play a role in physiological ageing [73]. Next to induction of apoptosis another way of responding to DNA damage is the process of terminal differentiation at the loss of self renewal ability [6; 74; 75].

One such example is the removal of melanocyte stem cells via differentiation into mature melanocytes in the niche after irreparable DNA damage. The result is the induction of hair graying, a typical sign of ageing, which can be further enhanced by depletion of ATM [75]. Patients suffering from AT, display a number of age-related phenotypes, including premature hair graying, but also hematopoietic abnormalities, resulting from impairments in the pool of hematopoietic stem cells and neurological defects [76]. Furthermore, DNA damage-induced, p53-mediated differentiation has been shown for neuronal stem cells in the subventricular zone [77] and the quiescent state and self renewal ability of hematopoietic stem cells can be actively regulated by p53 and ATM [6].

According to the cancer stem cell hypothesis, tumors, similar to normal tissues are maintained by the function of (cancer) stem cells [78]. Cancer stem cells have been shown to be more resistant to DNA damage-inducing therapy than the bulk of the tumor in various studies, including a number of different resistance mechanism, such as upregulation of DNA repair, drug transporters or modulation of survival pathways [5; 79; 80].

DNA damage and ageing

DNA damage is thought to play a crucial role in ageing. Inherited progeria syndromes such as Cockayne or Werner syndrome, as well as AT are a result of defective DNA repair or DDR signaling genes [1; 55; 81]. Although progeria syndromes share many features with physiological ageing, not all phenotypes can be extrapolated [73]. Nevertheless, also normal ageing seems to be, at least in part a result of accumulation of lesions during the lifetime of an organism, presenting a stochastic deterioration [82]. The response to DNA damage must play a dual role; ensuring on the one hand the avoidance of cancer formation, but on the other hand maintaining tissue integrity by keeping the stem cell pool intact. The “antagonistic pleiotropy” theory reflects this cellular dilemma, indicating that especially tumor suppressor genes such as p53 or mTOR which are beneficial for avoiding cancer formation in early life are implicated in ageing in later life [83]. In recent reports it was illustrated that the suppression of p53 target genes p21 and PUMA can

lead to a short term improvement survival in a telomere-dysfunction-induced progeria model, which was related to increased stem cell maintenance. However, suppression of PUMA-induced apoptosis will - over time - result in accumulation of DNA damage which eventually leads to induction of cell cycle arrest by p21 [84].

DNA damage-induced interference with housekeeping functions

DNA damage-induced signaling responses have to be integrated with ongoing cellular functions, such as transcription, translation and metabolic processes. Generally, DNA damage leads to reduced housekeeping functions, at the same time enhancing stress specific programs [85].

Bulky DNA lesions, crosslinks, DSBs, but also smaller lesions which are bound by mismatch repair proteins, can actively interfere with DNA transaction and stall replication forks and RNA polymerases [86]. Stalled RNA polymerase II functions as a damage sensor, initiating a cellular signaling response and leading to recruitment of (nucleotide excision) repair factors [3; 54]. Reduced transcription after (UV)-DNA damage has been linked to apoptosis and can be partly overcome by translesion transcription, an antiapoptotic mechanism in which RNA polymerase II continues transcription without prior repair of the lesions [87].

Also mRNA translation is frequently affected by DNA damage induction, with a general trend to attenuate global translation, while often favoring translation of DDR specific transcripts [88; 89; 90]. Various mechanisms can lead to translational repression, including the inhibition of translation initiation, interference with methionyl t-RNA recruitment or block of elongation [91; 92]. Eukaryotic translation initiation mostly depends on the 5´cap structure which is required for ribosome recruitment and launch of translation. Many translation repressive mechanisms act on the cap binding protein eiF4E. eiF4E stability and activity can be regulated on transcriptional and posttranscriptional level, and is targeted by inhibitory interactors, such as eiF4E-binding proteins (eiF4BPs) [91]. eiF4BP1 is a phosphorylation target of mTOR and Akt and can adjust metabolic and proliferation status of the cell to the amount of translation [89; 92]. Inhibition of mTOR-mediated phosphorylation by Rapamycin is used in cancer therapy [93].

Interestingly, after DNA damage often cap-independent translation initiation is required to increase levels of DDR specific proteins, such as the translation of a number of nucleotide excision repair factors, which relies on upstream ORFs in the 5' UTRs of the mRNA transcripts after UV damage; or IRES-mediated p53 translation after damage [88; 94]. Mechanism of stress-induced translation inhibition are frequently disabled in cancer cells, which contributes to the enhanced growth requirements of tumors, but also results in enhanced levels of endoplasmatic reticulum stress [92].

DNA damage in the context of cancer formation and treatment

DNA damage and cancer formation

Mutations caused by exogenous sources of DNA damage have been closely linked to the incidence of certain cancers, such as cigarette smoke-related lung cancer

development or skin cancer related to an excess of UV exposure [5]. Furthermore, genomic instability is an inherent feature of tumors. The importance of faithful genome maintenance is reflected in inherited cancer susceptibility disorders, which are linked to DNA repair or DDR signaling genes. These include the MMR-associated syndrome hereditary non-polyposis colorectal cancer (HNPCC or lynch syndrome), hereditary breast and ovarian cancer incidence linked to the deficiency in the HR genes BRCA1 and BRCA2, skin cancer susceptibility syndromes linked to defects in NER such as XP and syndromes cause by mutations in DDR signaling molecules such as AT (caused by mutations in ATM) and Li Fraumeni (caused by mutations in p53 or Chk2) [1; 11; 51; 95]. Also in spontaneously arising tumors there is a high rate of genomic instability, which is described as one of the hallmarks of cancer [96; 97]. The consequence of the continuous DNA damage in tumors is the accumulation of mutations. One distinguishes driver mutations in crucial genes, which determine the malignant changes within the tumor and so called passenger mutations that arise as a result of the constant DNA damage; however, have little or no impact on the physiology of the tumor [95].

The elevated occurrence of genomic instability in cancers is a result of shortening of telomeres, which provides a site for chromosomal fusion events, as well as of oncogenic stress, resulting in subsequent replication stress [98]. In the early neoplastic stage there is often a continuous activation of the DDR, serving as a barrier against

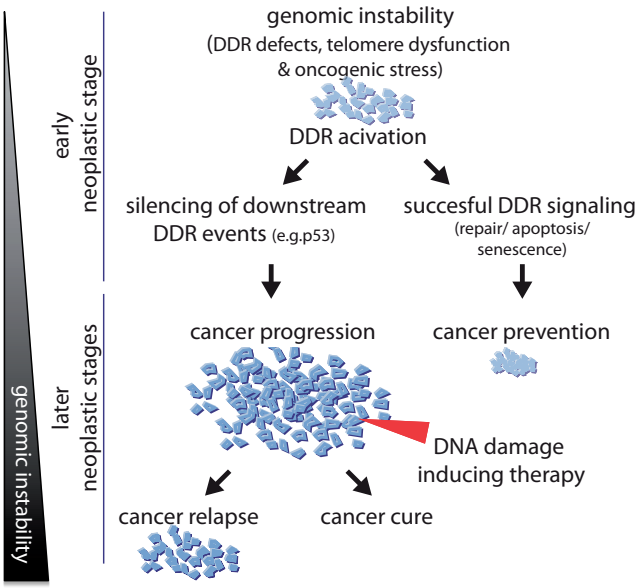

Figure 2. Genomic instability in cancer formation and treatment. At early neoplastic stages tumors show genomic instability due to DDR defects, telomere dysfunction or oncogenic stress. Activation of the DDR can act as a barrier against cancer formation. However, if the tumor progresses downstream signaling events are often silenced. This leads to an increase in genomic instability and mutation rate in the tumor. DNA damage inducing therapy can lead to cure of the cancer, but if relapse occurs, the tumor will likely show even more genomic instability.

further transformation. At later neoplastic stages, the early signs of the DSB response such as γH2AX and 53BP1 foci persist, whereas the signaling response, which in many cases would lead to p53-mediated induction of apoptosis is lost [99]. Furthermore at later stages, tumor cells, which have been subjected to DNA damage inducing cancer therapy, show enhanced genomic instability as a result of the treatment itself [51] (Fig 2). Genomic instability in cancers is not only seen as a hallmark of cancer initiation and progression, but can also serve as a predictive or prognostic biomarker. HR-deficient tumors, such as BRCA1 and BRCA2-deficient breast and ovarian cancers have been shown to be highly responsive to platinum agents [51].

Genetic screens might prove useful for identification of the genes that are crucial drivers in cancer formation and novel genes which might predict treatment responses [100]. However, not only gene deletion but also other mechanisms of silencing such as promoter methylation and posttranscriptional mechanisms of regulation can lead to tumor phenotypes, such as the one observed for BRCA1 and BRCA2 inactivation. An interesting alternative to studying gene expression, might lay in the determination of DNA repair capacity (e.g. by studying formation of Rad51 DSB repair foci) in patient cells, to predict the treatment response [51].

Exploiting the DDR for improved cancer therapy

Treatment of cancers often exploits DNA damage pathways. Radiation, but also chemotherapeutic drugs such as alkylating agents, antimetabolics and topoisomerase poisons are used to induce DNA damage, eventually leading to cell death in cancer cells [101]. Tumor cell inherent features such as a high proliferation rate and internal genomic instability make them more susceptible to genotoxic treatments than untransformed cells of the human body. However, toxicity of chemotherapeutics or radiation for healthy tissue and primary or acquired resistance constitute rate-limiting factors for the success of DNA damage inducing cancer therapy. Furthermore, genomic instability in cancers is a double-edged sword, since on the one hand it makes tumor cells more sensitive to DNA-damaging cancer therapy but on the other hand the high mutational rate allows for acquiring resistance by selecting for mutations that favor survival in the presence of DNA damage. This phenomenon is reflected in the behavior of BRAC1-deficient ovarian cancers, which initially respond well to platinum compounds, but over the course of treatment acquire resistance, often by genetically or epigenetically reintroducing the mutated DNA repair gene [100].

Clarifying the mechanisms which determine the DDR is therefore of outmost importance to improve the efficacy of therapy. The goal hereby is to specifically kill cancer cells, while sparing cells of healthy tissues from the harmful effects of DNA damage, preferably by taking into account inter-patient and potentially inter-tumor genetic variability, as well as differences in the microenvironment of tumors and healthy tissues, such as hypoxia and protective effects of stromal cells within the tumor [14; 51; 95; 102; 103].

To find novel genetic interactions, which meet the requirements for targeted therapy, research has focused on synthetic lethality approaches, a concept adopted from yeast genetics, where the deletion of one gene alone has no effect whereas the combination of two gene deletions can lead to decreased cell survival. The hope is to specifically kill cancer cells, by exploiting their deficiencies in DNA repair pathways or G1/S checkpoint activation (which makes them depend more heavily on the G2/M checkpoint) [103]. The so far most potent application of this concept was found in the relation between the inhibition of the ribosylase PARP and players of the DSB repair pathways i.e. tumors bearing mutations in the repair factors BRCA1 and BRCA2, which are involved in hereditary breast cancer [104; 105]. Inhibition of PARP causes an excess of single strand breaks, leading to secondary DSBs. While those can be repaired by HR in normal cells, HR-deficient cells will be killed specifically. Inhibitors of other DDR factors such as Chk1 and DNA-PK are currently investigated in clinical studies [95]. Although the targeting of key players of the DDR has been proven to be beneficial in some cases it also bears risks, since those factors often evoke plethoric responses activating not only apoptotic pathways but also DNA repair, cell cycle arrest or other prosurvival responses and may therefore lead to undesired effects, depending on the genetic context of a cell, tumor or patient. This has been demonstrated by ambiguous clinical responses to ATM and Chk2 inhibitors [14; 35].

AIMS AND OUTLINE OF THIS THESIS

Cellular responses to DNA damage are highly variable and strongly depend on the cellular and organismic context. Studying the DNA damage response is crucial for a better understanding of cancer formation and ageing as well as genotoxic stress-induced cancer therapy. To do justice to the multifaceted cellular changes, elicited by DNA damage, use of high-throughput techniques and integration with bioinformatics tools is of great value.

In this thesis I summarize recent advances in the field of systems biology studies of the DDR **(Chapter II)**. Furthermore, I show integrated approaches of the study of DDR signaling networks in embryonic stem and cancer cells (**Chapter III-VI)**. **Chapter III** focuses on the integration of transcriptional changes and the phosphorylation response of cisplatin-treated ES cells, identifying an induction of Wnt signaling as a crucial modulator of cell killing. In **Chapter IV**, RNAi screens for the cellular ubiquitination machinery identify the E3 ubiquitin ligase ARIH1 as a mediator of DNA damage-induced translation arrest, which acts as a prosurvival response in stem cells and cancer cells. **Chapter V** combines metabolomics profiling and transcriptomics analyses of cisplatin treated ES cells, identifying crucial metabolic pathways in the ES cell DDR. Genes, whose knockdown sensitizes ES cells to DNA damage-induced killing, are tested in cancer cells of varying genetic backgrounds in **Chapter VI**, identifying a small subset of genes, which represent potential drug targets for sensitization of cancer cells, which lack active p53- or caspase-3-mediated apoptosis.

Finally in **Chapter VII**, our findings are discussed and a proposal for future research lines is indicated. Altogether, our systems approach for studying the DDR identifies novel DNA damage-induced signaling networks and molecules, which modulate survival in the presence of DNA damage, potentially providing new targets for therapeutic intervention or biomarker discovery.

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