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Review

Importance of DNA damage checkpoints in the pathogenesis of human cancers

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ABSTRACT

All forms of life on earth must cope with constant exposure to DNA-damaging agents that may promote cancer development. As a biological barrier, known as DNA damage response (DDR), cells are provided with both DNA repair mechanisms and highly conserved cell cycle checkpoints. The latter are responsible for the control of cell cycle phase progression with ATM, ATR, Chk1, and Chk2 as the main signaling molecules, thus dealing with both endogenous and exogenous sources of DNA damage. As cell cycle checkpoint and also DNA repair genes, such as BRCA1 and BRCA2, are frequently mutated, we here discuss their fundamental roles in the pathogenesis of human cancers. Importantly, as current evidence also suggests a role of MAPK's (mitogen activated protein kinases) in cell cycle checkpoint control, we describe in this review both the ATR/ATM-Chk1/Chk2 signaling pathways as well as the regulation of cell cycle checkpoints by MAPK's as molecular mechanisms in DDR, and how their dysfunction is related to cancer development. Moreover, since damage to DNA might be the common underlying mechanism for the positive outcome of chemotherapy, we also discuss targeting anticancer treatments on cell cycle checkpoints as an important issue emerging in drug discovery.

Contents

Introduction

In the last two decades, substantial progress has been made in understanding the molecular pathogenesis of cancer [52], while growing knowledge paves the way for a better control of human cancer diseases. Increasing accumulation of alterations in oncogenes and tumor suppressor genes, as well as dysfunctions in the DNA damage response (DDR): (i) in the control of cell cycle checkpoints and (ii) in the DNA repair system, are hallmarks of carcinogenesis and thus important factors in the pathogenesis of malignant tumors. The well-studied DNA damage sensor p53 has mainly been recognized as a central factor in the G_1/S checkpoint [80,148]. However, importantly, in the last several years, our knowledge of the molecular organization of the DNA damage response network has meanwhile gone beyond the functions of p53, and is unraveling its whole complexity [20,104,115,161]. Against this background, it is not astonishing that alterations in the DNA damage response network and cancer also have found increasing interest [9,11,45,65]. In this review, we will at first briefly describe the current knowledge of the molecular functions of the DNA damage response network, placing emphasis on the role of their alterations in specific human cancer entities. Furthermore, we will discuss those cancer entities in which alterations in the DDR-system are of particular importance.

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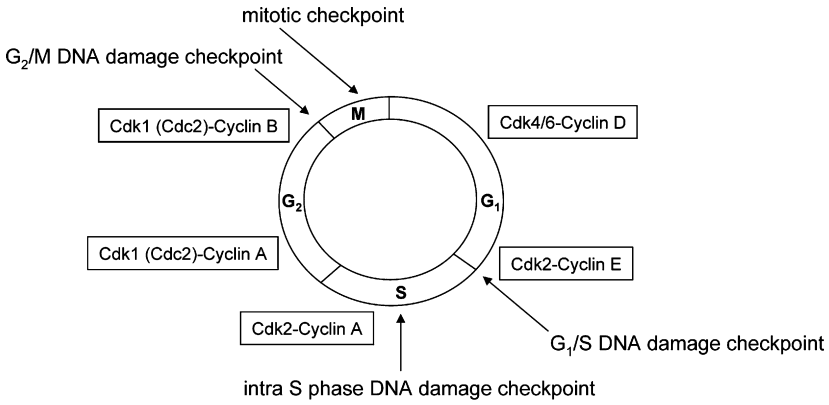

Fig. 1. Control of the cell division cycle by cyclins and Cdks and their regulation by cell cycle checkpoints. Details can be found in the text.

Molecular biology of DDR signaling pathways and their role in carcinogenesis

Due to the basic principles of oxidative life, the DNA molecule is a constant target of endogenous cellular metabolites, such as reactive oxygen species (ROS), an inevitable byproduct of aerobic life [112]. Moreover, different kinds of exogenous DNA-damaging factors, such as ultraviolet light, ionizing radiation, and a large number of organic and anorganic chemical substances, attack DNA. As ROS may cause different alterations in the genome, such as simple DNA mutations, DNA single and double strand breaks (SSB, DSB), or more complex changes, including deletions, translocations and fusions, the conventional view on ROS in carcinogenesis mainly focuses on DNA damage. Furthermore, DNA polymerase makes mistakes in the rate of $1{:}10^9$ during replication. Conceptually, while the DNA molecule is a constant target of damaging agents, the organisms essentially possess DNA damage checkpoints as an anticancer barrier for maintaining the correct transmission of genetic information from one cell to the next one [95].

The control of the mammalian cell cycle division is subjected to numerous Cdk (cyclin-dependent kinase)—cyclin complexes (Fig. 1). In the early G_1 phase of the cell cycle, Cdk4/6–Cyclin D complexes are active. Subsequently, entry into and progression through S phase are regulated by Cdk2–Cyclin E and Cdk2–Cyclin A, respectively, while the onset of mitosis is governed by Cdk1 (Cdc2)–Cyclin B. In addition, the activities of Cdk's are inhibited by numerous cyclin-dependent kinase inhibitors, such as $p21^{WAF1}$. Due to the existing cell cycle phases G_1, S, G_2, and mitosis, the transition points G_1/S, G_2/M, as well as the intra-S phase and mitotic checkpoint, are particularly tightly controlled checkpoints [10,115] (Fig. 1). They arrest or delay cell cycle progression in response to DNA damage. Initially, DNA damage checkpoints were defined as critical points in the cell cycle, where the cycle could be arrested until damage repair [53,152]. In the last several years, a growing body of evidence has revealed more complex functions of the DNA damage checkpoints, how they comprise the entire arsenal of cellular responses to DNA damage. Thus, DDR acts on several axes: it regulates cell cycle arrest, activates DNA repair mechanisms, induces cell cycle death by apoptosis, regulates telomere length, and activates critical transcriptional programs [1,102]. In addition, it could also be shown that some checkpoint genes are essential for the survival of organisms causing embryonic lethality in mice if the particular gene is knocked out [14,83,137]. This confirms that checkpoint genes not only regulate DNA damage but, in addition, have essential functions in cellular survival.

As damage to cellular DNA is considered the most important and initiating factor in the development of cancer, epidemiologic studies confirm a direct relationship between factors damaging the DNA molecule and the development of cancer [30]. Consequently, cellular responses to DNA damage and hence cell cycle checkpoint responses are key issues in the pathogenesis of cancer. The last several years have seen many studies that investigated the factors of DNA damage response and how their dysfunction can induce cancer diseases [45,65]. Therefore, to understand human cancers, it is of paramount importance to accumulate knowledge of the biology of DDR. Importantly, because DDR acts on several cell responses, including cell cycle arrest, DNA repair, or apoptosis, also the dysfunction of DDR may originate from one or more of these cell responses, suggesting a comprehensive role of DDR in carcinogenesis. In fact, DDR dysfunctions have been found to play a role in the pathogenesis of practically all common human cancer entities [65]. Furthermore, alterations in the molecules of DDR are in the spotlight of investigations regarding the pathogenesis of cancer susceptibility syndromes [2]. An example is the well-known association between inherited mutations of the DNA repair enzymes BRCA1 and BRCA2 and breast or ovarian cancer [147].

In summary, the importance of DDR function and dysfunction in oncology also arouses the interest of pathologists. As the DNA damage checkpoints are highly conserved in evolution with much overlap between mammals and yeast, we here concentrate on the types of molecules that can be found in mammals.

Organization of DNA damage checkpoint responses

DNA damage checkpoint responses are organized as signal transduction pathways. Therefore, firstly, the damage is recognized by sensors, and secondly, the signals are transmitted to mediators and transducers. The transducer molecules suppress effector kinases or phosphatases, including proteins involved in transcriptional regulation, DNA repair, and cell cycle control, such as p53 and Cdc25. Certain molecules may have different functions in this signal transduction pathway [104]. For example, ATM and ATR can simultaneously act as sensor and transducer, and BRCA1 may also have multiple functions [158]. Consequently, the signal transduction in DNA damage response is not one-dimensional but a complex network of interacting molecules. Thus, to give a comprehensive practical overview, we here describe in particular those factors that have been proved important to the pathogenesis of human tumors.

Sensor-recognized signals rapidly activate different molecules with transducer function. Among these, ATM and ATR are of major importance. These very large proteins, with a molecular weight of 350 kDa, phosphorylate different substrates in the DDR signaling networks. ATM and ATR belong to a conserved family of proteins that are characterized by a domain with motifs of the lipid kinase phosphatidylinositol-3-kinase (PI3K), [128].

ATM is one of the master controllers in DNA damage response, especially when DSB have occurred. Originally, it was discovered as a gene responsible for the human genetic disorder ataxia–telangiectasia (A–T). Therefore, it was given the name ATM "ataxia–telangiectasia-mutated" [118]. A–T is inherited in an autosomal recessive manner at a frequency of 1:40,000–1:100,000 live births. Many different mutations of the ATM gene have been described in A–T. In the majority of cases, the ATM protein underlies truncations, whereas amino acid substitutions are less frequently described. The cellular phenotype is characterized by an abnormal response to irradiation and by genomic instability [78]. A–T is clinically characterized by immune deficiencies, loss of motoring control as a result of Purkinje cell loss, and a high frequency of cancers. The changing role of ataxia–telangiectasia from a rare disorder to an important paradigm for cell signaling and cancer has recently been pointed out [77].

In its role as a transducer, ATM activates multiple proteins in a special protein complex to achieve its functional goals. In the last few years, many ATM substrates and hence downstream targets have been elucidated. Here, only the key activities of ATM can be mentioned. The direct activation of the p53 protein by ATM was described at first [43]. Furthermore, ATM preferentially activates the checkpoint protein kinase Chk2, thereby enhancing its kinase activity [94,161]. However, there can also be some crosstalk with Chk1 [65]. The activated Chk2, in turn, phosphorylates p53, thereby inhibiting the binding of Mdm2 to p53 with the result that p53 protein is not targeted for degradation and is therefore stabilized. Via this pathway, p53 is also indirectly activated by ATM, while activated p53, in turn, can navigate cells to apoptosis or to cell cycle arrest in the G_1 phase (Fig. 2A). Hence, signal transduction via ATM has a central function in guiding cells to cell cycle arrest or to apoptosis. Besides p53 and Chk2, BRCA1 has also been reported as an ATM target [26]. Therefore, it can also be phosphorylated and activated by ATM. It is localized in a large protein complex with many other proteins that have important functions in DNA damage response [89]. Additional to its functions in DDR, ATM is also involved in the regulation of oxidative stress by interacting with the antioxidative enzyme systems [63].

ATR, another molecule with an important transducer function in cell cycle checkpoints orchestrating cellular response to DNA damage, was discovered as a gene with sequence homology to ATM and to Rad3 (ataxia–telangiectasia- and Rad3-related). As a consequence of sequence homology, the function of ATR is partly redundant with that of ATM. Thus, substrate specificities of ATR and ATM are partially overlapping. Interestingly, Tibbetts et al. suggest that ATM and ATR function sequentially with ATM in the early and ATR in the late stages of DNA damage response [141,163]. Recently, a preferred binding of the ATR protein to UV-damaged DNA was observed [144]. In general, Chk1 is the preferred ATR substrate, while Chk2 is the favored ATM target [46,85,160]. The functional differences between ATM and ATR could be confirmed by a different behavior of ATM and ATR null mice. ATM null mice are viable with a phenotype characterized by infertility and growth retardation [7,36]. In contrast, ATR null mice die in early embryogenesis, exhibiting a phenotype resembling mitotic catastrophe [14,29]. Obviously, ATR is essential for life most probably due to its additional functions in monitoring DNA replication [24].

Chk1 and Chk2 are protein kinases that have important functions in different cell cycle checkpoints and are activated by upstream sensors and transducers within the checkpoints. Being activated, Chk1 and Chk2 phosphorylate downstream targets of the cell cycle checkpoints network. Although Chk1 and Chk2 have many similar functions, their proteins are structurally unrelated except the protein kinase domain [11]. In principal, increased ATM activates the downstream kinase Chk2 and the tumor suppressor gene p53 [111], while Chk1 is activated by ATR-mediated phosphorylation (Fig. 2).

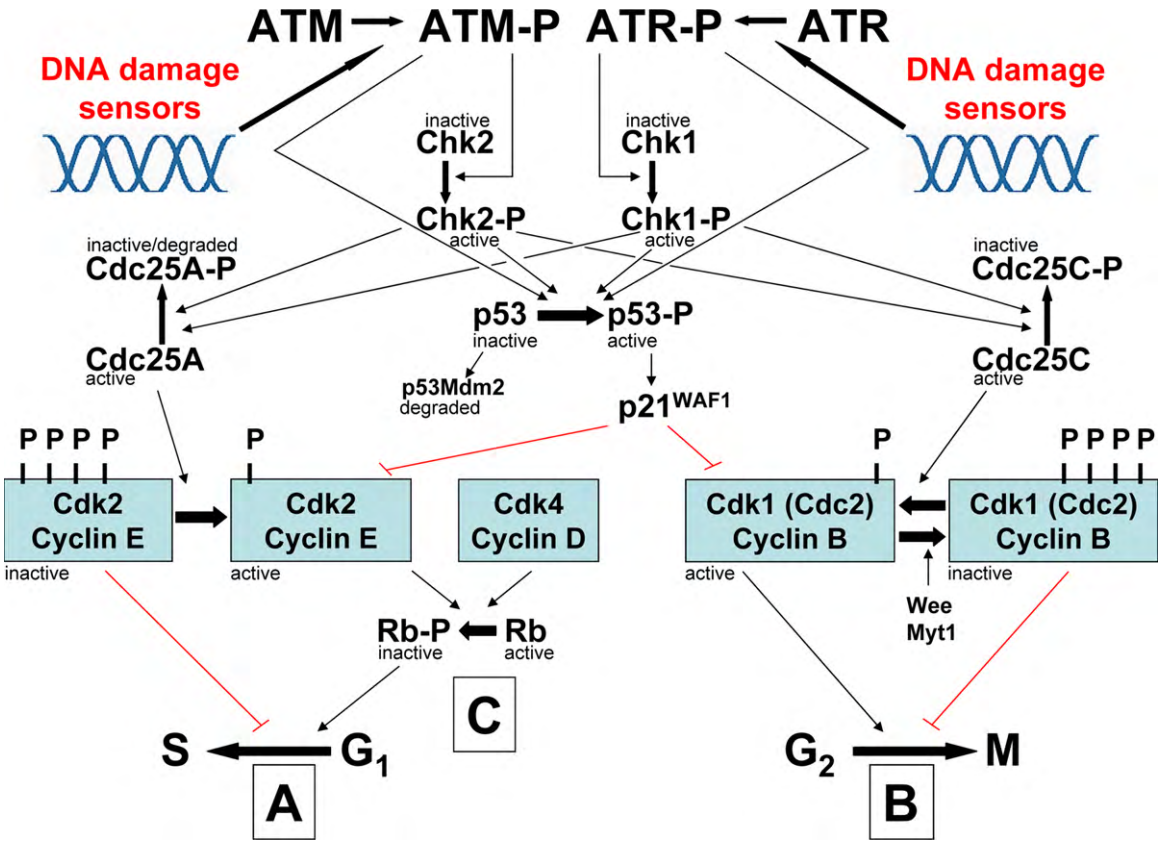

Fig. 2. The G_1/S and G_2/M checkpoint regulation network following upstream ATM/ATR activation. Details can be found in the text.

For this phosphorylation, the mediator protein claspin is needed [76,82]. However, the Chk1 phosphorylation does not appear to regulate kinase activity of Chk1, but rather its subcellular localization. Thus, phosphorylated Chk1 tends to translocate to centrosomers where it prevents premature activation of Cdk1 (Cdc2)–Cyclin B1 through inhibition of Cdc25C [59,74,103]. More recently, a role of Chk1 in the M transition and spindle checkpoint has been reported [135,159].

It must be mentioned that the networks are not so simple that ATM can activate Chk2 and ATR Chk1. Instead, there is a high degree of redundancy in these DNA damage pathways. Furthermore, the functions of Chk1 and Chk2 are multiple. When they are activated by ATR and ATM following DNA damage, they can influence many different cellular processes such as circadian rhythm, chromatin remodeling, and the regulation of transcription [111]. Chk1 is an essential gene that is required for normal embryonic development. Thus, Chk1 knock out mice are not viable [83,125,137]. In contrast, Chk2 knockout mice are viable, and mutations have been found in a growing number of human malignant tumors [50]. Given the roles of Chk1 and Chk2 in cell cycle regulation, they may, in future, also serve as targets for therapeutic inhibition [4].

Cdc25 phosphatases are important key targets of Chk1 and Chk2 [5]. The Cdc25 gene is highly conserved and can already be found in fission yeast. In mammalians, three isoforms are known, each with different functions: Cdc25A, Cdc25B, and Cdc25C. Cdc25A controls progression through S phase and mitosis, while Cdc25B and Cdc25C control mitotic entry [32]. Cdc25B and Cdc25C null mice show a normal development. This indicates the redundancy of the Cdc25 isoforms in mammalians [21,40]. Kristjansdottir and Rudolph showed that Cdc25 phosphatases are overexpressed in different human cancer entities [75].

Description of the ATR/ATM-Chk1/Chk2 signaling pathways in DDR

In general, one key function of Chk1 and Chk2 activated by ATR and ATM, respectively, manifests in the inactivation of different members of the Cdc25 family by phosphorylation, resulting in a stop of cell cycle progression after DNA damage in the G_1/S phase or the G_2/M phase. Basically, three main pathways can be described (Fig. 2).

(1) The G_1/S checkpoint is subject to the Chk1/Chk2–Cdc25A–Cdk2 pathway (Fig. 2A)

The active unphosphorylated Cdc25A phosphatase executes its function through dephosphorylation of the Cdk2–Cyclin E complex. As a consequence, the Cdk2–Cyclin E complex is kept in its active form, which causes progression of the cell cycle from G_1 to S. Following DNA damage, Chk1 and Chk2 phosphorylate Cdc25A, inducing its degradation. Due to the degradation of the Cdc25A phosphatase, the Cdk2–Cyclin E complex remains in its hyperphosphorylated inactive form, culminating in G_1/S arrest (Fig. 2A).

(2) The G_2/M checkpoint is subject to the Chk1/Chk2–Cdc25C–Cdk1 pathway (Fig. 2B)

Investigating the mechanism responsible for mitotic entry at the G_2/M checkpoint, it was found that activating dephosphorylation of only a small amount of Cdk1–Cyclin B1 is the initiating step for mitotic entry [57]. Thus, activated Cdk1–Cyclin B1 then activates Cdc25C, creating a positive feedback loop and leading to entry into mitosis [109,113]. In addition, Cdk1–Cyclin B1 can also inactivate Wee1, thereby forming two positive feedback loops [151].

Accordingly, mitosis-triggered Cdk1 (=Cdc2) is the ultimate target of the G_2 checkpoint regulation. Cdk1 is phosphorylated at two positions by protein kinases Wee1 and Myt1, and is dephosphorylated by Cdc25C phosphatase. Increased phosphorylation of Cdk1 by Wee1/Myt1 or prevention of its dephosphorylation by Cdc25C phosphatase triggered by activated Chk1 induces G_2/M DNA damage checkpoint arrest. Thus, the maintenance of the Cdk1–Cyclin B1 complex in its inactive state blocks entry into mitosis. In effect, the signal cascade after DNA damage via Chk1 and Chk2 can halt the cell cycle by controlling the Cdk1–Cyclin B1 complex via governing its activator Cdc25C (Fig. 2B).

(3) The Chk1/Chk2–p53–$p21^{WAF1}$–Cdk pathway is centered on p53 (Fig. 2B and C)

p53 has for long been known as a key player in governing the G_1/S checkpoint. In response to DNA damage, p53 can be phosphorylated at multiple sites by several different protein kinases [92,99]. The phosphorylation of p53 can be catalyzed by ATM, ATR, DNA-PK, and Chk1/Chk2. Phosphorylation impairs the ability of Mdm2 to bind p53, promoting both, the accumulation and activation of p53 in response to DNA damage [126,141]. In addition, the phosphorylation of p53 appears crucial in enhancing its transcriptional transactivation activity [34]. Activated p53 upregulates a number of target genes, several of which are also involved in DNA damage response, such as Gadd45 and $p21^{WAF1}$. The accumulation of $p21^{WAF1}$ suppresses Cdk2–Cyclin E, thereby resulting in G_1 arrest [9], (Fig. 2C). Thus, G1 arrest is a consequence of preventing Rb phosphorylation via inhibition of Cdk2, repressing the release of the G1/S phase-promoting E2F transcription factor. On the other hand, p53 also has functions in the G_2M checkpoint: p53 activated via Chk1/Chk2 may trigger induction of $p21^{WAF1}$, which blocks the formation of the mitotic Cdk1–Cyclin B1 complex and inhibits its activity [131,133,139], (Fig. 2B).

In summary, during checkpoint control following DNA damage, p53 can either be phosphorylated directly by ATM or ATR [6,18,51,141], or indirectly by ATM and ATR via Chk1 and Chk2 [55,125]. Certain cancer-related mutations in the Chk2 gene can prevent phosphorylation of p53 [37,61]. The effects of Chk1 and Chk2 in the regulation of p53 also depend on the site where p53 is phosphorylated. However, a detailed description of the multitude of phosphorylation sites of p53 and their importance for this activation is beyond the scope of this review.

Regulation of the cell cycle checkpoints by MAPK's

Importantly, besides Chk1 and Chk2, the MAPK (mitogen activated protein kinases) family has become increasingly important in cell cycle control in response to DNA damage [155]. As a consequence, the important DDR pathways are connected with the important MAPK pathway and thus, the MAPK pathway also has functions in DNA damage response. The MAPK pathway is activated after a variety of cellular stimuli and regulates numerous physiological processes, in particular the cell division cycle.

(1) Role of p38 MAPK in cell cycle checkpoints (Fig. 3)

p38 MAPK can regulate both the G_1/S and the G_2/M cell cycle checkpoint in response to DNA injury [140]. p38 activation contributes to the induction of a G_1/S checkpoint by multiple distinct mechanisms, including phosphorylation of p53 and even $p21^{WAF1}$, phosphorylation and stabilization of the transcriptional repressor HMG-box protein 1 (HBP1), direct phosphorylation of Cyclin D1 for proteasomal degradation, phosphorylating the Cdc25A phosphatase, promoting its degradation, and up-regulating p16INK4a and p19ARF gene expression (Fig. 3A).

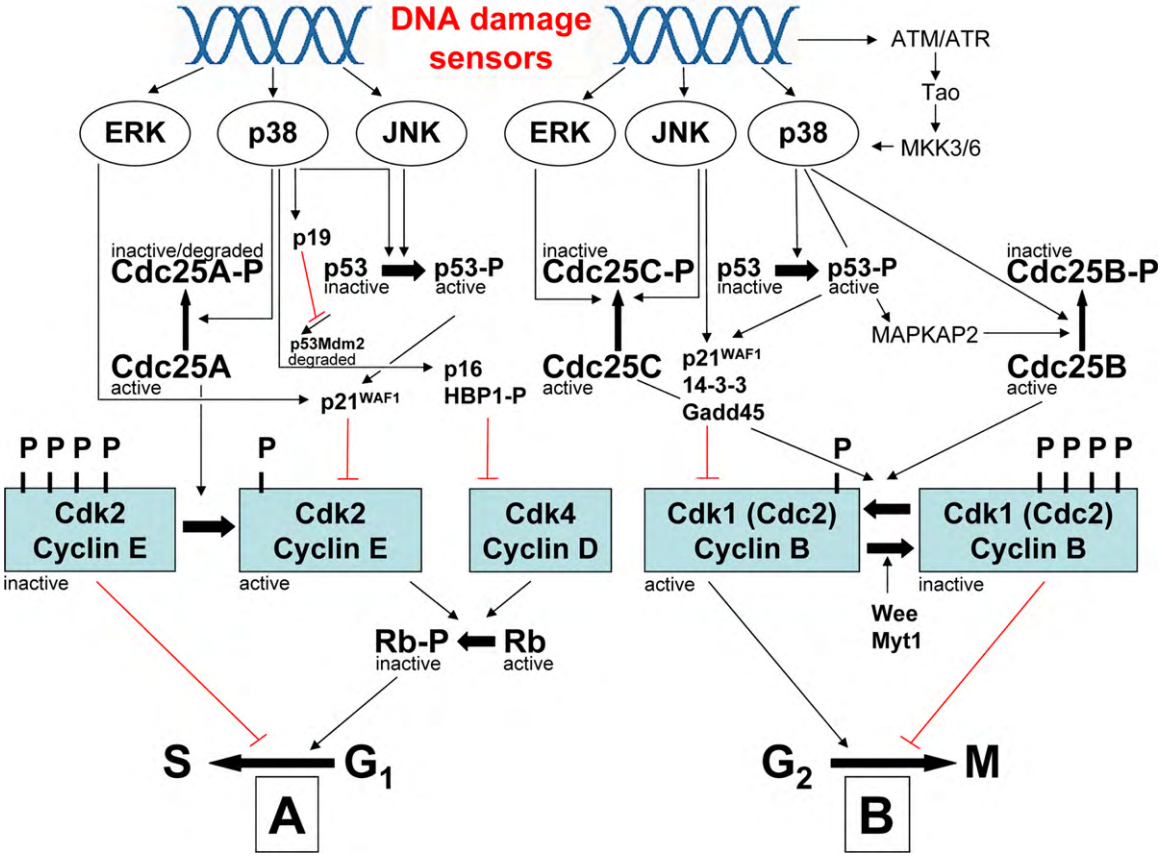

Fig. 3. The G_1/S and G_2/M checkpoint regulation network following upstream MAPK activation. Details can be found in the text.

In the G_2/M checkpoint, p38 MAPK activation occurs indirectly through ATM and ATR kinases. In addition to the ATM/ATR-dependent pathway, p38 MAPK can also be activated by other not yet fully established mechanisms. However, once activated, p38 induces G_2/M cell cycle arrest (i) via phosphorylation of p53, which induces target genes, such as Gadd45 and $p21^{WAF1}$ or (ii) through direct phosphorylation and inhibition of the Cdc25B phosphatase [140], (Fig. 3B). Thus, the p38 MAPK not only acts via p53 [16,44,81,90,97,98,107]. The etiologic factors that activate the p38 MAPK pathway to phosphorylate and inactivate the Cdc25 phosphatases are similar to those of the ATM/ATR pathway. Furthermore, recent results have shown that the phosphorylation of Cdc25 isoforms by p38 MAPK may also proceed, though not directly. Instead, p38 MAPK activates the MAPK-associated protein 2 (MAPKAP2), which phosphorylates Cdc25 isoforms [88]. This new inactivation pathway of Cdc25 isoforms by p38 MAPK and MAPKAP kinase-2 shows that Cdc25 isoforms are a target of different major activation and inactivation pathways in the molecular circuit of the mammalian cell. Probably, more effectors of checkpoint pathways will be discovered in the years to come.

(2) Role of JNK MAPK in cell cycle checkpoints (Fig. 3)

JNK function in cell cycle control may be restricted to mature tissues and environmental stress, including mitogens, cytokines, DNA-damaging agents, and cellular stress. In addition to p38, JNK may participate in DNA damage-induced G_1/S checkpoint control by phosphorylating p53, which led to G_1/S arrest [17], (Fig. 3A). Furthermore, JNK has been implicated in the basal regulation of the G_1/S phase transition, S phase progression, and mitosis under non-stressed conditions [71,157]. Recently, Gutierrez et al. reported that JNK plays a central role in cell cycle control for both proper G_2/M transition under non-stressed growth conditions and G_2/M checkpoint arrest following DNA damage through JNK-dependent regulation of Cdc25C activity by phosphorylation on Ser168 [47], (Fig. 3B). Furthermore, JNK-mediated G_2/M arrest has also been reported to occur through JNK-involved induction of $p21^{WAF1}$ expression following oxidative stress [64,122].

(3) Role of ERK MAPK in cell cycle checkpoints (Fig. 3)

ERK has been well established in the regulation of basal G_1 to S phase and S phase progressions. Furthermore, activation of ERK mediates sustained $p21^{WAF1}$ expression, which results in G_1 arrest [23], (Fig. 3A). In addition, centromeric ERK has been reported to possibly play a role in spindle assembly checkpoint pathways [22,62]. Remarkably, ERK regulates Cdc25C via phosphorylation during the G_2/M transition in mitotic and meiotic cells [150] (Fig. 3B).

Practical importance of dysfunctions in the DDR network for human cancer diseases

This chapter briefly summarizes the reasons why dysfunction of the DDR network is practically important for human cancers. The main aspects are as follows:

(1) A link between defective DNA damage response and pathogenesis of cancer has been known for a long time [9,56,128]. Accordingly, alterations in the genes of checkpoint kinases have been observed in a large variety of common and less common malignant human tumor entities.

(2) In the last few years, DNA damage response has been recognized as an anticancer barrier in human tumors, and a constitutive activation of DNA damage signaling has been shown with increased expression of the checkpoint kinases ATM, ATR, Chk1, Chk2, and H2AX, particularly in their phosphorylated activated

forms [9]. A high degree of activation has been reported even in early human precancerous lesions [9,45,49].
(3) The majority of therapeutic agents currently used for cancer treatment, including chemotherapeutic substances and radiation therapy, aim at damaging cellular DNA. Therefore, some authors presume that it might be possible to sensitize cancers to therapeutic DNA-damaging agents by inhibiting important checkpoint kinases via chemical inhibitors [60,139].
(4) Genetic alterations in checkpoint kinases play a major role in cancer susceptibility syndromes [2].
(5) For clinical purposes, it is also important that DNA damage is the main reason for chemotherapeutic side effects such as bone marrow suppression, hair loss, and gastrointestinal incompatibilities [65].

Somatic *ATM* mutations have been considered important in human cancers. Heterozygous carriers of ATM mutations and increased cancer predisposition are still matters of controversy [68]. ATM mutations that cause ataxia–telangiectasia are considered as breast cancer susceptibility alleles [2]. An increase in ATM mutations has been reported in sporadic human tumors, mostly in tumors of the lymphatic system. It has been suggested that ATM behaves like a tumor suppressor gene in these malignancies [130]. Loss of ATM has been described as an important factor for the development of lymphomas [129]. In Mantle cell lymphoma, the DNA damage response pathway is abrogated by alterations in the ATM gene and less often by alterations in the downstream checkpoint kinases Chk1 and Chk2. This observation is also important from the practical point of view. It has been demonstrated that an expression profile of proliferation-associated ATM downstream genes has been proven as the strongest survival predictor in these tumors [41]. Immunohistologic investigations were conducted to demonstrate total ATM and phosphorylated ATM. These studies revealed that most human tissues mainly contained the non-phosphorylated inactive form, while the phosphorylated form could be detected in a subset of bone-marrow lymphocytes and in spermatocytes in the adult testis. A constitutive expression of phosphorylated ATM was also observed in human germ cell tumors, especially in embryonic carcinomas. Expression was weaker in seminomas [8]. Lu et al. showed that ATM haploinsufficiency enhances susceptibility to cancer-induced mammary tumors [87]. These authors treated a congenic strain of ATM +/− mice with DMBA (7,12-dimethylbenz-alpha-anthracene), a well-known carcinogen to induce mamma tumors. The ATM +/− mice had a twofold increase of malignant tumors and a threefold increase of mammary dysplasia when compared with carcinogen-treated wild-type mice. These studies confirm that ATM heterocygotes have an increased risk of developing mamma tumors. As it is assumed that 1% of the human population is heterozygote for ATM (A–T-carriers), the importance of this finding for clinical aspects of breast cancer patients is beyond doubt.

ATR mutations predispose to Seckel syndrome. Patients with this disease show dwarfism, mental retardation, and microcephalia [105]. In humans, an increased incidence of malignant tumors could not be observed in these patients, but it could be shown that haploinsufficiency of ATR in mice defective for DNA repair reveals enhanced tumorigenesis [39,65].

It has already been pointed out that complete loss of *Chk1* results in early embryonic lethality. The lethal consequences of Chk1 deficiency in mammals might explain why mutations of Chk1 are rare in human tumors. So far, they have been found only in rare cases of colon- stomach- and endometrium carcinomas [13,96,146]. However, it could be shown that a heterozygous state of Chk1 in wnt transgenic mice enhanced the tumorigenic phenotype of these animals [84]. In the same way, inactivating mutations of $p21^{WAF1}$ have not been identified in tumors, but its regulator *p53* is mutated in 50% of human tumors. However, it has been shown that cytoplasmic localization of $p21^{WAF1}$ is induced via phosphorylation, promoting cell survival [162]. Thus, the phosphorylation of cell cycle regulators seems to provide a novel mechanism of tumors to disrupt their function without gene mutation, and hence cellular proliferation is no longer under normal growth control.

In contrast to Chk1, *Chk2*-deficient mice are viable. They reveal increased tumor susceptibility when treated with carcinogen or in their later life [54,136]. First evidence of Chk2 alterations as pathogenetic factor in human cancers was obtained from unusual cases of Li–Fraumeni syndrome, which did not reveal the typical p53 alterations causing the vast majority of Li–Fraumeni syndromes. In these p53 wild-type Li–Fraumeni cases with predominance of breast cancer and different sarcomas, Chk2 alterations were observed [12]. Obviously, germ line mutations of Chk2 may be alternative genetic defects for Li–Fraumeni syndrome [11]. Particularly, the 1100delC variant of the Chk2 gene was associated with this cancer syndrome.

Genetic alterations of the *Chk2* gene have been found in different human tumor entities. A combined genetic and epigenetic analysis of Chk2 in sporadic primary breast- ovarian- and colon tumors showed that LOH across the Chk2 locus is common in these tumors. However, point mutation or epigenetic inactivation of the other allele is uncommon [153]. In addition, Chk2 expression was analyzed by immunohistochemistry in 119 ovarian cancers performed on tissue microarrays. Twenty-three percent of the cases were negative for Chk2 protein, and no correlation could be observed between LOH and Chk2 protein expression. To investigate if polymorphic Chk2 variants could play a role in the etiology of ovarian tumors, Szymanska-Pasternak et al. analyzed 539 women with benign ovarian cystadenomas, 122 women with borderline ovarian tumors, and 447 women with invasive ovarian cancer [134]. The results showed that Chk2 variants predispose to borderline tumors and low grade invasive ovarian cancers. Surprisingly, there was no correlation with high grade ovarian cancers.

The 1100delC variant of the Chk2 gene, which can preferentially be found in Li–Fraumeni syndrome, has been found to be a cancer susceptibility gene in hereditary breast cancer [93,145]. As the 1100delC variant of Chk2 occurs at a frequency of 1.1–1.4% in the North American and Western European population, the practical importance for human breast cancer patients is obvious [93,145]. Chk2 variants also predispose to prostatic cancer [27,31,123,134].

Another functionally defective Chk2 variant, I157T, has been associated with an increased risk of familial and sporadic colorectal cancer [70]. The frequency of Chk2 I157T was significantly higher in colorectal cancers than in the normal population. A recent study analyzed Chk1, Chk2, and their activated phosphorylated forms using Elisa and Western blot in a large number of colorectal carcinomas and normal colonic mucosa. Expression of Chk2 and phosphorylated Chk2 was found to be decreased in 50% of the tumors, indicating their inactivation as pathogenetic factor for colorectal carcinogenesis [132].

It has long been known that inheritance of a single mutated allele for *BRCA1* or *BRCA2* is associated with an increased risk of breast- and ovarian cancer [72]. BRCA1 has been established as a tumor suppressor [121], and Chk2 has been described as a component of the DNA damage signaling pathway activated in response to BRCA deficiency [91].

MAPK signaling pathways essentially regulate all aspects of malignant cell behavior, including cancer-related characteristics such as proliferation, survival, migration, and invasion. Thus, the role of MAPK's in cancer is as pleiotropic as cancer itself. Recent studies suggest that a decrease in *p38* activity plays an important role in cancer. For example, p38 activity has been shown to be reduced in hepatocellular carcinomas in comparison to adjacent normal tissue [58]. Besides its role in oxidative stress response, *JNK* activity and thus phosphorylation of c-jun has been reported to participate in Ras-induced tumorigenesis, and Ras and c-jun cooperate

in cellular transformation [66]. In this context, one important function of JNK/c-jun appears to be the transcriptional repression of the p53 gene [35,120]. In contrast to these findings, studies on JNK1/2-null cells have shown that JNK is not required for ras-induced transformation and tumorigenesis *in vivo*. Instead, JNK may promote apoptosis through its tumor suppressor function [67]. The *ERK* pathway is deregulated in approximately one-third of all human cancers. Conceptually, it is not astonishing that signaling via ERK is linked to aspects of the tumor phenotype, such as cell proliferation. Furthermore, most cancer-associated lesions with constitutive ERK activation also show for example overexpression of receptor tyrosine kinases, activating mutations of receptor tyrosine kinases, and *Ras* mutations. Thus, the pathway whereby growth factors and mitogens activate ERK is of particular importance to cancer. Importantly, high levels of ERK can lead to cell cycle arrest by inducing $p21^{WAF1}$ and p27 [101,124,154]. To continue to proliferate, certain tumor cells utilize elevated Rho signaling or constitutively activated Akt to abrogate the ERK-mediated induction of growth arrest [25,101,106,114].

Targeting DNA damage checkpoint kinases as anticancer treatment

Radiation therapy and chemotherapy have been used as important modalities for anti cancer treatment for decades. It is likely that they will remain in use for anti cancer therapy in the foreseeable future. Their central function is to damage DNA. The damaged DNA will immediately activate the DDR network, and thus hamper the desired effects, while cancer growth arrest and apoptosis are major outcomes desired in drug treatment. Since damage to DNA might be the common underlying mechanism for the positive outcome of anticancer therapy, an important issue emerging in drug discovery is to target anticancer treatments on cell cycle checkpoints. Recently, we could show that besides the epigenetic regulation of the $p21^{WAF1}$ promoter [48], G_2 checkpoint arrest is not only induced trough the checkpoint kinase Chk1, but early Chk1 activation also directs senescence and mitotic catastrophe – two main strategies to long-term affect the growth of tumor cells – even in recovery from G_2 checkpoint arrest [108]. Furthermore, inhibition of important molecules in the DNA damage response network has increasingly been considered as an innovative strategy for improving the effects of chemotherapy and radiation therapy [60,116,138]. Significant experience in this field was already gained in the 1990s. At that point of time, it could be shown that caffeine is an inhibitor of ATM and ATR [117]. However, the high dosage of caffeine required for this function cannot be applied to humans.

Chk1 with its central function in DNA damage response pathways has also been considered as an appropriate target for inhibition strategies. In the last several years, a large number of chemical inhibitors to Chk1 have been developed [60]. These substances inhibit, in addition to Chk1, a variety of kinases, including Chk2. The therapeutic possibilities of targeting Chk1 are discussed in a recent review [60]. The possibilities and also problems caused by applying Chk2 inhibitors as therapeutic approach in clinical oncology have recently been reviewed by Antoni et al. [4]. To optimize the effects of chemotherapy and radiation therapy, inhibitors to a larger number of other checkpoint kinases are also being developed.

In addition, because of its importance in cancer, the ERK pathway has been a focus for drug discovery, but rather with Ras, Raf, and MEK as the main targets [33,73]. Furthermore, inhibition of p38 activity causes enhanced apoptosis in response to DNA-damaging agents, such as doxorubicin and cisplatin, as well as microtubule-disrupting agents, such as taxol, vicristine, and vinblastine [28,79,86]. In this context, also JNK inhibitors have been considered for anticancer therapy, and this is linked to their ability to interfere with DNA repair in response to genotoxic drugs [67]. However, as JNK may also have a tumor suppressor function due to its ability to promote apoptosis, JNK inhibitors would then prevent apoptosis, and thus the usefulness of JNK inhibitors is still under discussion.

Conclusions

During the last several years, considerable progress has been made in elucidating the checkpoint pathways as part of the DNA damage response. As a consequence, the discovery of DNA damage checkpoints in G_1/S, S, and G_2/M considerably contributed to the debate on the role and regulation of the DDR network, especially the practical importance of its dysfunctions in human cancer disease, discussed in this review. Thus, key components of the DDR machinery, such as ATM, ATR, Chk1, Chk2, BRCA1, and BRCA2, were highlighted regarding their importance for human cancers. In general, alterations in the genes of these key components caused by mutation have been considered important in tumors of the lymphatic system (ATM, Chk2), in breast cancer (ATM, Chk2, BRCA1, BRCA2), ovarian cancer (Chk2, BRCA1, BRCA2), colon cancer (Chk2), prostatic cancer (Chk2), the Li–Fraumeni syndrome (Chk2), and the Seckel syndrome (ATR). In contrast, Chk1 mutations have been found only in rare cases of colon, stomach, and endometrium carcinomas. This is due to the lethal consequences of Chk1 alterations. In addition, besides mutations, the posttranslational phosphorylation of DDR components has also been shown to play a role in human tumors. For example, phosphorylated ATM has been detected in a subset of bone-marrow lymphocytes, in spermatocytes in the adult testis, and in embryonic carcinomas. Furthermore, phosphorylated ATM and Chk2 was found in preneoplasias of prostatic cancer. In addition, phosphorylated $p21^{WAF1}$ promotes the survival of HER-2/neu-overexpressing cells. Thus, the phosphorylation of DDR components seems to provide a novel mechanism of tumors to disrupt their function without gene mutation, and hence cellular proliferation is no longer under normal growth control.

Given the role of MAPK's in so many critical responses required for cellular homeostasis, it is not astonishing that loss of the fine control of MAPK regulation plays a role in human cancer disease. However, less is known about the question of how different environmental stresses which trigger the activation of MAPK pathways (JNK, ERK, p38) contribute to deregulated cell cycle progression in human cancer cells. Thus, to what extent the altered MAPK signaling is directly connected with MAPK's dysfunction in DDR and therefore with the pathogenesis of human cancer disease will be a central question in the years to come.

Malignant human tumors with alterations of DDR molecules as important pathogenetic factor

Breast cancer

Inherited breast cancer is associated with germline mutations in different genes critical to genomic integrity and particularly DDR [149]. As has been known for longer time, BRCA1 and BRCA2 mutations confer a very high risk of breast- and also ovarian cancer. Among others, mutations in Chk2 and ATM are associated with doubling of breast cancer risk [15]. In Chk2, especially the 100delC mutation has revealed increased susceptibility to breast cancer [42,93]. A link between breast cancer and A–T was recognized more than 20 years ago, when it could be shown that relatives of A–T patients have an increased risk of developing breast cancer [100].

Prostate cancer

ATM has been shown to be important in prostate cancer and particularly in its prestages. Fan et al. found immunohistologically increased phosphorylated ATM and phosphorylated Chk2 in preneoplasias of prostatic cancer such as prostatic intraepithelial neoplasia (PIN) [38]. Obviously, activation of ATM and Chk2 suppresses tumor progression in prostatic

carcinogenesis. Common polymorphisms in the ATM gene could be established as risk factors for prostate cancer development [3,37]. Cesaretti et al. found out that possession of sequence variants in the ATM gene, particularly those that encode for an amino acid substitution, predict the development of adverse radio-therapy responses among patients treated with 125I prostate bradytherapy [19].

Lung cancer

Yang et al. presented data which provide the first epidemiologic evidence that genetic variants of ATM may effect non-small lung cancer (NSCLC) predisposition, and that the risk-conferring variants might act through down regulating the function of ATM in DNA repair activity upon genetic insults such as ionizing radiation [156]. On the other hand, Schneider et al. found ATM variants in 16% of the German control subjects and in about 14% of lung cancer patients [119]. Thus, ATM gene mutations had no detectable modifying effect on lung cancer risk in their study.

Gynecologic tumors

Szymanska-Pasternak et al. analyzed 539 women with benign ovarian cystadenomas, 122 women with borderline ovarian malignancies, and 447 women with invasive ovarian cancer for variants of the Chk2 gene [134]. The Chk2 I157T missense variant was increased in cystadenomas, borderline tumors, and low grade invasive cancer, but, astonishingly, not in high grade ovarian cancers. Williams et al. analyzed the expression of Chk2 by immunohistochemistry in 119 ovarian tumors and combined the results with genetic and epigenetic analysis [153]. Twenty-three percent of ovarian tumors were negative for Chk2 protein, but there was no significant correlation between LOH across the Chk2 locus and intensity of Chk2 staining. Reddy et al. analyzed Chk2 in vulval neoplasia [110]. BRCA1 and BRCA2 mutations confer a high risk for developing ovarian cancer [149].

Gastrointestinal cancers

The expression of Chk1 and Chk2 and their phosphorylated active forms (pChk1 and pChk2) in colorectal cancers and normal colonic mucosa was analyzed by Stawinska et al. using Western blot and Elisa [132]. A detailed analysis of the Chk2 variant 1100delC was given by Kilpivaara et al. [69]. Menoyo et al. found somatic mutations of ATR and Chk1 in sporadic stomach tumors with microsatellite instability [96]. Furthermore, Shigeishi et al. described an increased expression of Chk2 in gastric carcinomas harboring p53 mutations [127]. Bertoni et al. found Chk1 frameshift mutations in genetically unstable colorectal and endometrial cancers [13].

Hematologic neoplasias

Tort et al. showed that Chk2- decreased protein expression and infrequent genetic alterations occur in aggressive types of non-Hodgkin lymphomas [142]. Furthermore, Chk1 protein and mRNA expression was down regulated in aggressive variants of human lymphoid neoplasms [143]. In Mantle cell lymphoma, the DDR pathway was shown to be affected by frequent alterations in the ATM gene as well as Chk1 and Chk2.

Germ cell tumors

Using an antibody against phosphorylated ATM, Bartkova et al. analyzed normal human tissues and also testicular germ cell tumors [8]. pATM was observed especially in embryonal carcinomas, less frequently in seminomas, and only modestly in teratomas and in preinvasive carcinoma in situ stage.

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