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REVIEW

DNA Damage Response Genes and the Development of Cancer Metastasis

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DNA damage response genes play vital roles in the maintenance of a healthy genome. Defects in cell cycle checkpoint and DNA repair genes, especially mutation or aberrant downregulation, are associated with a wide spectrum of human disease, including a predisposition to the development of neurodegenerative conditions and cancer. On the other hand, upregulation of DNA damage response and repair genes can also cause cancer, as well as increase resistance of cancer cells to DNA damaging therapy. In recent years, it has become evident that many of the genes involved in DNA damage repair have additional roles in tumorigenesis, most prominently by acting as transcriptional (co-) factors. Although defects in these genes are causally connected to tumor initiation, their role in tumor progression is more controversial and it seems to depend on tumor type. In some tumors like melanoma, cell cycle checkpoint/DNA repair gene upregulation is associated with tumor metastasis, whereas in a number of other cancers the opposite has been observed. Several genes that participate in the DNA damage response, such as *RAD9*, *PARP1*, *BRCAl*, *ATM* and *TP53* have been associated with metastasis by a number of *in vitro* biochemical and cellular assays, by examining human tumor specimens by immunohistochemistry or by DNA genome-wide gene expression profiling. Many of these genes act as transcriptional effectors to regulate other genes implicated in the pathogenesis of cancer. Furthermore, they are aberrantly expressed in numerous human tumors and are causally related to tumorigenesis. However, whether the DNA damage repair function of these genes is required to promote metastasis or another activity is responsible (e.g., transcription control) has not been determined. Importantly, despite some compelling *in vitro* evidence, investigations are still needed to demonstrate the role of cell cycle checkpoint and DNA repair genes in regulating metastatic phenotypes *in vivo*. © 2014 by Radiation Research Society

INTRODUCTION

DNA is continuously damaged by genotoxic agents generated either in the environment (e.g., UV light, ionizing radiation, etc.) or intracellularly (e.g., reactive oxygen species as byproducts of routine metabolic processes). In normal cells, the integrity of the genome is ensured by a very efficient DNA damage response signaling network that includes cell cycle checkpoints and DNA repair pathways. On the other hand, cancer cells are thought to arise through the accumulation of numerous genetic alterations that confer growth and survival advantages. Dysregulation (either loss or gain) of DNA repair factors can promote the accumulation of DNA errors and genomic instability, which is implicated in aging, immune deficiencies, neurodegenerative disorders and cancer. Germline mutations in cell cycle checkpoint or DNA repair genes can predispose to hereditary forms of cancer, whereas somatic mutations and epigenetic silencing of DNA damage response genes are common in cancers with no inherent genetic link (1). DNA repair genes involved in nucleotide excision repair (2), mismatch repair (3), non-homologous end joining (4, 5) and homologous recombination (6) can predispose to different types of cancer.

Dysregulation of DNA repair genes affects the response of cells to DNA damaging anti-cancer treatment. Upregulation of DNA repair pathways can cause resistance to chemotherapy and radiotherapy, so inhibitors of these pathways have the potential to sensitize cancer cells to these agents (7, 8). Conversely, cancer cells that have lost a repair pathway and are solely dependent on another, alternative pathway, can be rendered vulnerable by targeting the functional pathway using the principle of synthetic lethality, whereas noncancer cells (with two functional repair pathways) would demonstrate resistance (7, 8).

In recent years, there has been accumulating evidence that DNA damage response genes are involved in additional cellular functions beyond mending damaged DNA and cell cycle checkpoint control, such as transcriptional regulation, chromatin remodeling and apoptosis. In this article, we provide an overview of the evidence that DNA damage

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response (DDR) genes participate not only in tumor initiation, at least in part when they fail to ensure proper repair of damaged DNA, but in tumor progression and metastasis as well.

DNA REPAIR GENES AND CANCER INITIATION

Genomic instability is characteristic of most human malignancies and it is considered a hallmark of cancer cells. Genomic instability is caused by downregulating DNA damage response pathways, such as those controlled by p53, ataxia telangiectasia mutated (ATM) and AT and Rad3-related (ATR) kinases. Alternatively, genomically unstable tumors can arise from acquired defects in any one of six DNA repair or damage tolerance pathways, base excision repair (BER), nucleotide excision repair (NER), DNA mismatch repair (MMR), homologous recombination repair (HR), non-homologous end joining (NHEJ), and translesion DNA synthesis (TLS). The connection between DNA repair defects and carcinogenesis is highlighted by the fact that inherited defects in DNA repair mechanisms that cause progeroid or accelerated aging syndromes (9), including Ataxia telangiectasia, Nijmegen syndrome, Werner syndrome, Bloom syndrome, Rothmund-Thomson syndrome, Xeroderma pigmentosum or Trichothiodystrophy also carry a higher cancer risk as well (2, 10).

In addition to DNA damage response gene mutations, which can either be inherited or somatically acquired, epigenetic gene silencing may also promote tumorigenesis. Epigenetic inactivation of DNA repair genes in cancer has been reported and has been related to several DNA repair pathways including BER, NER and other DNA damage processing mechanisms (11). In sporadic cancers, one of the most common mechanisms of inactivation of DNA repair pathways is the epigenetic silencing of a critical gene (e.g., *FANCF*, *BRCA1*) through methylation of the promoter region. Epigenetic silencing of DNA repair genes, such as *MGMT*, *MLH1*, *BRCA1*, *WRN* and *FANCF*, can boost mutation rates and promote genomic instability in cancer cells (12). Below is a brief description of the connection between specific aberrant DNA repair pathways and human cancer.

Direct Repair

The simplest form of DNA repair is the direct reversal of a lesion. *MGMT* (O⁶-Methylguanine DNA methyltransferase) participates in this kind of pathway and is often at higher levels in tumors compared with normal cells where it can confer resistance to DNA-alkylating agents. The *MGMT* enzyme repairs O⁶-alkylated guanine residues in genomic DNA. O⁶-methylguanine pairs with thymine and would lead to a G-to-A transition during DNA replication if left unrepaired. *MGMT* is epigenetically silenced (13) in a variety of tumors, including glioblastoma (14–16), colon cancer (17, 18), non-small cell lung cancer (19, 20), gastric

carcinoma (21), and head and neck squamous cell carcinoma (22–24). In the case of colorectal cancer, *MGMT* promoter methylation is associated with G-to-A mutations in *KRAS* (25) and in p53 (26).

Base Excision Repair

The base excision repair (BER) pathway is the main mechanism that protects the genome from deleterious effects of exposure to reactive oxygen species. This pathway removes damaged bases from DNA and it can also repair DNA single-strand breaks (27). A number of DNA glycosylases (e.g., *OGG1*: 8-oxoguanine DNA glycosylase; *MUTYH*: the human homolog of the *E. coli mutY* gene), endonucleases (*APE1*: apurinic/apyrimidinic endonuclease 1; *FEN1*: flap structure-specific endonuclease 1), *XRCC1* (X-ray repair complementing defective repair 1), DNA polymerase β (*Pol* β), DNA ligase III and *PARP-1* participate in this DNA repair pathway.

Inherited mutations in BER genes are rare. However, polymorphisms in genes like *OGG1*, *APE1* and *XRCC1* have been genetically linked to cancer (28). *MUTYH*, (29) is the first BER gene to have been associated with a human cancer syndrome (*MUTYH*-associated polyposis), as biallelic germline mutations in *MUTYH* were identified in individuals with a pre-disposition to multiple colorectal adenomas and carcinomas (30, 31). Other DNA glycosylases are also found dysregulated in various cancers. For example, *OGG1*, which repairs oxidatively damaged guanine bases in DNA, is involved in tumorigenesis (32–34), whereas expression of the thymine DNA glycosylase gene (*TDG*) is decreased in several multiple myeloma cell lines compared with normal plasma cells by promoter methylation (35). Production of *APE1*, an enzyme that follows the action of DNA glycosylases in the BER pathway, is frequently increased in germ cell tumors (36), and higher *APE1* protein levels have been associated with increased drug and radiation resistance (36, 37). Further downstream in the BER pathway, *Pol* β , which is the major DNA polymerase that fills in the nucleotide gap created by *APE1*, is overexpressed in prostate, ovary, uterus and stomach cancers (38), as well as prostate, breast and colon cancer cell lines (39). In addition, elevated levels of *Pol* β lead to genomic instability through the accumulation of DNA single- and double-strand breaks (40, 41), and these effects are particularly evident after exposure of *Pol* β -overexpressing cells to oxidative stress-inducing DNA damaging agents (42).

FEN1, which participates in BER (43), non-homologous end joining (44) and homologous recombination (45), and is important for genomic stability (46), demonstrates increased expression in many tumors. It is highly abundant in testis, lung and brain tumors (47) and in prostate cancer (48), metastatic prostate cancer cells (49), neuroblastomas (50) and pancreatic cancer (51). In breast cancer, this

increase is due to the absence of *FEN1* promoter methylation (52).

Finally, glioblastoma cells overexpressing EGFRvIII, an oncogenic variant of epidermal growth factor receptor (EGFR), become hyper-dependent on a variety of DNA repair genes (53), including an enrichment of base excision repair genes required for repair of reactive oxygen species-induced DNA damage. One example is PARP-1 (53), which generally shows higher abundance in tumors. Besides PARP-1, other BER enzymes (TDG, OGG1) were also upregulated in EGFRvIII-containing cells after radiation exposure. The increased reliance on BER in these cells suggests the presence of elevated reactive oxygen species levels abundance cells with resultant genomic instability.

Nucleotide Excision Repair

The nucleotide excision repair (NER) pathway is a major DNA repair process that safeguards genome integrity by repairing numerous DNA modifications, especially bulky helix-distorting damage (54). However, recent work has revealed that some proteins in NER have activities that go beyond DNA repair and include nucleosome remodeling, histone ubiquitylation, and transcriptional activation of genes involved in nuclear receptor signaling, stem cell reprogramming and post-natal mammalian growth (55).

Tumors with enhanced NER have an intrinsic resistance to radiotherapy and chemotherapy (56), leading to continued growth and metastasis after treatment (57). On the other hand, NER is often disrupted in testicular germ cell tumors due to loss of *XPA* expression (58). Likewise, *XPC*, a critical component of global genome NER, is controlled by promoter methylation in bladder cancer (59). In addition, *ERCC1* is also inactivated through promoter methylation in glioma tumors (60). Moreover, mutations of *XP* (xeroderma pigmentosum complementation group) *A*, *B*, *C*, *E*, *F* and *G* have been found in skin and testicular cancer, and variant expression of *ERCC1* (excision repair complementation group 1) or *XPD* was demonstrated in lung cancer (61).

Mismatch Repair

Mismatch repair (MMR) targets incorrectly paired nucleotides introduced accidentally by DNA polymerases or after treatment with base-modifying chemotherapeutic drugs (e.g., alkylating agents) (62). MMR disruption causes microsatellite instability (MSI), a form of genetic instability associated with cancer. Familial cases of colonic tumors with MSI in Lynch syndrome result from germline mutations in mismatch repair genes, primarily *MSH2* and *MLH1* (63). However, most MSI-high tumors arise from an epigenetic defect in sporadic cases of cancer (64). Methylation in the promoter region of *MLH1* correlates with decreased activity in sporadic colon cancer (65, 66). Likewise, *MLH1* is also controlled by aberrant methylation in sporadic endometrial carcinoma (67), gastric cancer (68) and many other cancers.

Homologous Recombination

DNA double-strand breaks (DSBs) pose the most serious threat among all genotoxic assaults to the survival of cells and are repaired by either homologous recombination (HR) or non-homologous end joining (NHEJ). HR is disrupted in breast and ovarian cancer (69). *BRCA1* and *BRCA2*, two important players in the HR pathway, are mutated in early onset breast and ovarian cancer (70), prostatic (71) and pancreatic cancer (72). Loss of *BRCA1* expression by promoter hypermethylation is also seen in nonhereditary breast and ovarian cancer (73). In addition, the gene that encodes NBS1, which along with MRE11 and RAD50 constitute a heterotrimeric complex that senses DNA damage mainly in the form of double-strand breaks (74), is often mutated in lymphoma (Nijmegen breakage syndrome) (75). However, loss of *NBS1* expression is found in prostate cancer (76). Additionally, *RAD50* frameshift mutations, which result in a truncated protein, occur in a third of gastrointestinal cancers (77). The Fanconi Anemia/BRCA pathway, which repairs DNA crosslinks (74), is often impaired in a number of hematogenous and solid tumors. Thus, homozygous mutation of numerous FA genes (*A*, *B*, *C*, *D1*, *E*, *F*, *G*, *I*, *J*, *L*, *M* and *BRCA2*) or heterozygous mutation of some FA genes (e.g., *FANCA*, *FANCC*, *FANCG* and *BRCA2*) has been shown in hereditary breast, ovarian, cervical, prostatic, lung, pancreatic, gastric cancers, as well as melanoma and leukemia (78).

Non-homologous End Joining

DSBs are predominantly repaired by non-homologous end joining (NHEJ). The gene encoding DNA ligase IV, a major mediator of this pathway, is mutated in leukemia (Lig4 syndrome) (5), whereas *Artemis*, a structure-specific endonuclease, is mutated in lymphoma (Omenn syndrome) (4). Loss of *Ku70* expression in cervical, rectal and colon cancer has also been reported (79–81), whereas *Ku86* protein abundance is reduced in some rectal cancers (80).

Translesion DNA Synthesis

The translesion synthesis (TLS) machinery bypasses DNA adducts during DNA replication with the help of low stringency DNA polymerases (β , ι , κ). As mentioned above, *Pol β* is overexpressed in prostate, ovary, uterus and stomach cancers (38), whereas *Pol ι* is overexpressed in breast cancer (82) and *Pol κ* is overexpressed in lung cancer (83). Elevation in expression and activity of the error-prone polymerase, *Pol β* , accounts for the increase in cisplatin resistance and mutagenesis of many cancers (84).

DNA DAMAGE INDUCED CELL CYCLE CHECKPOINT MECHANISMS

Cell cycle checkpoints are activated to arrest transiently cell proliferation, allowing extra time to repair DNA

damage. When lesions are repaired, cells resume cell cycle progression (checkpoint recovery). However, when damage is irreparable cells either remain permanently arrested (senescence) or undergo programmed cell death (74). Defects in cell cycle related DNA damage response pathways result in genome instability and lead to carcinogenesis. Central to the DNA damage response are members of the phosphoinositide 3-kinase (PI3K)-related protein kinase family, ATM, ATR (ATM and Rad3-related), and DNA-PKcs (DNA protein kinase catalytic subunit) that sense the damage, and amplify the signal by phosphorylating numerous downstream substrates, including checkpoint kinases 1 and 2 (74). Activation of the upstream kinases require recognition of the damage. For DSBs, this is achieved by the heterotrimeric MRE11-RAD50-NBS1 (MRN) complex that directly binds to the exposed ends of DNA, recruits ATM and initiates its activation (85). Active ATM phosphorylates histone variant H2AX, which serves as a docking site for MDC1 (86) and many other proteins, including 53BP1, RNF8, RNF168 and BRCA1 (87).

Single-stranded DNA lesions generated from stalled replication forks are rapidly coated by replication protein A complexes, which then recruit ATR and its binding partner ATRIP (88), and independently Rad17 that in turn recruits the heterotrimeric RAD9-RAD1-HUS1 (9-1-1) complex to the site of damage. Subsequently, ATR phosphorylates the 9-1-1 complex, then RAD9 binds TopBP1 and RHINO (89, 90), which enhance ATR activity. Active ATR phosphorylates many substrates, including CHK1, an event important for establishing the cell cycle checkpoint (91).

Transition between the different phases of the cell cycle is dependent on cyclin-dependent kinases. Their activity is negatively regulated by WEE1 and MYT1 kinase mediated phosphorylation and, conversely, they become activated by the CDC25 phosphatases that dephosphorylate the inhibitory phosphorylations (92). After DNA damage, CHK1 induces CDC25A degradation by phosphorylating CDC25A and targeting it for proteosomal degradation (93). Alternatively, CHK1 activates WEE1 or NEK11 that further phosphorylates CDC25A, again preparing it for degradation (94). CHK2, although redundant for checkpoint activation in p53-proficient cells, becomes important for IR-induced cell cycle arrest in p53-deficient cells (95). Another mechanism implicated in G₁ checkpoint induction is rapid degradation of cyclin D1 and release of cyclin-dependent kinase inhibitor p21^{waf1/cip1}, culminating in cdk2/cyclin E inhibition and blocking G₁/S transition (96). Cyclin D1 destruction is induced by GSK3 β phosphorylation and SCF-dependent proteosomal degradation (97) or direct phosphorylation of the F-box protein FBXO31 by ATM (98).

CHK1 is crucial for maintaining genomic stability as it is required for monitoring replication fork progression during S phase of the cell cycle, and inhibition of CHK1 leads to stalling of replication forks and irreversible fork collapse (99-101). Furthermore, CHK1 also influences many aspects

of mitosis by controlling cyclin B/Cdk1 activation, contributing to spindle checkpoint function, chromosome segregation and cytokinesis (102-104).

Tumor suppressor p53 plays an important role in cell cycle checkpoints. Following genotoxic stress, ATM/ATR/DNA-PKcs and CHK1/2 phosphorylate as well as stabilize p53. Subsequently, p53 drives a transcription program that includes p21^{waf1/cip1}, which plays a pivotal role to initiate G₁ and sustain G₂ arrest (105, 106). Furthermore, p53 transcriptionally represses expression of cyclin B, CDC25B and polo-like kinase 1 (Plk1) that are required for mitotic entry (107-109). p53 also participates in DNA repair and it is the main factor that determines the choice between DNA damage repair or the induction of senescence or apoptosis.

CELL CYCLE CHECKPOINT CONTROL AND CANCER

The cell cycle response to DNA damage is a highly efficient barrier against tumorigenesis. Premalignant cells have to overcome this barrier to progress into more malignant states (110, 111). Failure of mechanisms that regulate DNA damage checkpoint control leads to chromosomal aberrations (112) and genomic instability (113), both of which contribute to neoplastic transformation (114). Furthermore, alterations of proteins that control the DNA damage response-signaling pathway and impair the stringency of cell cycle checkpoints create a permissive environment that allows mutations to accumulate. The rapid accumulation of mutations in the genome of a cell leads to the so-called "mutator phenotype", which contributes to tumor progression by creating tumor heterogeneity and subsequent emergence of aggressive types of cancer (115). The importance of an intact checkpoint related DDR mechanism is further underscored by the existence of hereditary cancer predisposition syndromes that are the result of a germ line mutation in a DDR gene, e.g., ataxia telangiectasia mutated (ATM), ataxia telangiectasia-like disorder (MRE11), Nijmegen breakage syndrome (NBS1), hereditary breast/ovarian cancer (BRCA1/2), Fanconi anemia (FA pathway genes) and Li-Fraumeni syndrome (TP53) (116). As mentioned previously, sporadic cancers have at least one defect in the DDR pathway, manifested as an altered cell cycle profile or sensitivity/resistance to genotoxic stress (7, 8), illustrating the importance of the DDR signaling in tumorigenesis.

Haploinsufficiency for a variety of DDR proteins, including ATM, ATR, γ -H2AX and CHK1 (117-121) or knockdown/knockout of NBS1 (122, 123), RPA (124, 125), RAD17 (126), BRCA1 (127) and BRCA2 (128) is associated with genomic instability. Combined ATM and RAD9 (129) and ATM and HUS1 (130) haploinsufficiency led also to increased genomic instability. ATR and CHK1 participate in HR repair (131-133), besides regulating the S and G₂ checkpoints and replication initiation and fork stability

(134–139), thus facilitating repair of DNA breaks and preserving genomic integrity. CHK2 is an important mediator of cell cycle checkpoints, DNA repair and apoptosis. CHK2 is usually absent or downregulated in non-small cell lung cancer (140). Heterozygous mutations in *ATR* and *CHK1* have been found in a subset of stomach, colon and endometrial cancers (141–143). In animal studies, *ATR* and *CHK1* hypomorphic mutations could contribute to increased risk of tumorigenesis (118, 144).

Other members of the DDR network, such as PLK1 (145), and Aurora kinases (146) are frequently overexpressed in human tumors and promote chromosomal instability through defects in the spindle assembly checkpoint that result in chromosome missegregation and centrosome amplification. In addition, cyclin-dependent kinases (CDK) are overactive in many tumors and a number of proteins that enhance or reduce CDK activation display oncogenic or tumor suppressive traits, respectively (147). CDC25 phosphatase (148), which is required for CDK activation, can act as an oncogene, whereas WEE1 that opposes the activation of CDK1 and enforces G₂/M arrest after DNA damage acts as a tumor suppressor (149, 150). Moreover, dysregulation of cyclin E is considered a major factor of tumorigenesis. Elevated abundance of cyclin E is associated with various neoplasias (151) and its prolonged expression induces chromosomal instability (152). A number of CDK-specific inhibitors are currently being tested in clinical trials for the treatment of patients with a variety of cancers, such as multiple myeloma, pancreatic, and lung carcinomas (147).

ROLE OF CELL CYCLE CHECKPOINT CONTROL AND DNA REPAIR GENES IN METASTASIS

Metastasis is the most clinically important attribute of cancer as more than 90% of cancer-related deaths are due to lack of local control. Tumor metastasis consists of a series of complex steps that need to be executed successfully to give rise to detectable tumors at sites distal to the organs where primary tumors initiate (153). Large-scale analyses of gene expression profiles of human cancers have revealed aberrant expression patterns of a number of genes involved in cell adhesion, migration, angiogenesis, kinase activation and other tumor-related functions. Although there is ample evidence that DNA repair genes are associated with the onset of tumorigenesis and DNA repair gene deficiencies cause inherent predisposition to cancer, a direct role of DNA repair or cell cycle checkpoint proteins in the etiology of metastasis has not been shown conclusively. Currently, the role of DNA repair genes in metastasis has only been inferred by DNA gene expression microarray analyses or by *in vitro* assays that serve as surrogates for *in vivo* metastasis phenotypes. Some of these genes are downregulated as tumors progress to a more malignant stage, while other genes are actually overexpressed and can affect the

metastatic process. Whether these genes control the metastatic process due to their role in DNA repair or cell cycle control and the maintenance of genomic stability or because of some novel functions (e.g., acting as transcription factors or co-factors, or by more direct involvement for example in cell adhesion to matrix, etc.) is currently not known.

A genome-wide screen that compared gene expression in metastatic prostate and primary prostate tumors identified a strong correlation between high proliferation rates in metastatic cancers and overexpression of genes that participate in cell cycle regulation, DNA replication and DNA repair (49). Oncomine analyses showed that numerous human cancers, such as of the prostate, brain, cervix, head and neck, kidney, bladder and pancreas, displayed elevated levels of DNA repair proteins [for review see ref. (154)]. Moreover, in melanoma, there is considerable evidence that DNA repair genes are upregulated in metastases compared with primary tumors (57, 155). Gene expression microarray analyses as well as immunohistochemical examination of human melanoma specimens have shown an increase in expression of genes involved in HR and NER, but not BER (155). In contrast to the progression from melanocytes to primary melanoma, genetic stability appears to be necessary for a melanoma cell to give rise to distant metastasis (156). Therefore, the majority of neoplastic cells, found in primary melanomas poised to metastasize, have overexpressed genes responsible for efficient repair, ultimately resulting in genetically stable cells that are able to metastasize and grow at distant sites (155). Based on these results, it has been hypothesized that genomic instability is beneficial for the early stages of tumor development, whereas advanced and metastatic tumors overexpress an array of DNA repair genes to ensure a minimum of genomic stability (157). This inactivation–activation mode of DNA repair genes is not without precedent in other contexts (e.g., chemoresistance). It has been shown experimentally that a repair pathway may become inactivated early in carcinogenesis resulting in chromosomal instability, whereas consequent secondary mutations confer a selective advantage to the tumor. Subsequently, the repair pathway is reactivated (78). A case in point is the loss of the Fanconi anemia-BRCA pathway in cisplatin-sensitive ovarian cancer and re-gain of the pathway activity after prolonged treatment with cisplatin, which results in resistance to the drug (158).

A number of genes that participate in DNA damage induced checkpoints and DNA repair, and are either upregulated (e.g., *RAD9*, *PARP1*) or downregulated/mutated (e.g., *BRCA1/2*, *ATM* and *TP53*) have been associated with metastasis that was demonstrated using a variety of *in vitro* assays and by examining human tumor specimens by immunohistochemistry. A review of some of these genes and their potential relationship to metastasis follows.

RAD9 Activities

RAD9 can function as part of a heterotrimer with RAD1 and HUS1 (the RAD9-RAD1-HUS1 complex, 9-1-1), which is recruited to DNA damage sites by the RAD17-RFC (replication factor C) complex and is required for the subsequent activation of CHK1 and cell cycle arrest (159). However, RAD9 is a versatile protein that participates in numerous cell functions besides cell cycle checkpoint activation, such as DNA repair, telomere maintenance, dNTP biosynthesis, apoptosis and transcriptional regulation of genes (160). In addition, RAD9 can interact with several other proteins outside the context of the 9-1-1 complex and checkpoint functions (160).

Human RAD9 is involved in almost all aspects of DNA repair, including base excision repair (161, 162), nucleotide excision repair (163), mismatch repair (164) and homologous recombination, but not non-homologous end joining (165). Telomere instability and ionizing radiation sensitivity are linked to defective DNA repair (166) and RAD9 affects both. Moreover, chromosome end-to-end associations have been connected to genomic instability and carcinogenesis (167–169). When RAD9 is inactivated, increases in chromosome end-to-end associations and frequency of telomere loss are observed (165). Studies in murine embryonic stem cells lacking *Mrad9* (*Mrad9*^{-/-} ES cells) demonstrate a marked increase in spontaneous chromosome aberrations (an increase in the frequency of chromosome and chromatid breaks) and *HPRT* (hypoxanthine phosphoribosyl transferase) mutations even in the absence of exposure to exogenous DNA damaging agents, indicating a role in the maintenance of genomic integrity (170).

RAD9 functions in apoptosis, in addition to its role in cell cycle checkpoint control and DNA damage repair pathways. *Mrad9* deficiency causes midgestational embryonic death, accompanied by increased apoptosis and reduced cellular proliferation (170). The lack of *Mrad9* in mouse ES cells also causes enhanced spontaneous apoptosis (171). RAD9 can interact and neutralize the action of anti-apoptotic Bcl-XL and Bcl-2 (172), and induce pro-apoptotic Bax activation (173). The pro-apoptotic action of RAD9 is potentiated by c-Abl phosphorylation (174) and protein kinase C delta (175), as well as the p63 transcription factor (176).

Another important and largely unexplored activity of RAD9 is its ability to function as a transcription factor and regulate a number of downstream target genes, most notably *p21^{waf1/Cip1}* (177). Human RAD9 has also been identified as a coregulator that can suppress androgen-androgen receptor transactivation in prostate cancer cell lines (178).

The Role of RAD9 in Metastasis

Reduction in RAD9 levels is associated with genomic instability manifested as telomere dysfunction, aberrant chromosomal segregation, high spontaneous levels of mutations, as well as defective DNA repair. Thus, given

the roles of RAD9 in maintaining genomic stability, it is reasonable to hypothesize that the protein is important for tumorigenesis. Indeed, studies by a number of laboratories have linked aberrations in RAD9 abundance to a variety of cancers or an impact on phenotypes representing hallmark features characteristic of neoplastic transformation (160). Aberrant RAD9 expression has been associated with breast, lung, skin, thyroid and gastric cancers. RAD9 is frequently overexpressed in human prostate cancer tissue specimens as well as prostate cancer cell lines and, importantly, down-regulation of RAD9 in human tumor cell line xenografts impairs growth in nude mice, thus establishing a causative role for RAD9 in prostate cancer (179).

Cancer metastasis is a multi-step process in which tumor cells progressively acquire traits, including detachment from the extracellular matrix, anoikis resistance (defined as resistance to cell death triggered when cells lose adhesion to extracellular matrix), migration and invasion through the basement membrane, intravasation to blood and lymphatic vessels, extravasation from the circulation to distant sites, the ability to stimulate angiogenesis, and, finally, formation of macroscopic secondary malignant growths. At the molecular level, a number of signaling pathways, including those that involve integrins and Akt, contribute to the survival and progression of a tumor. Integrins are heterodimeric $\alpha\beta$ transmembrane receptors that connect the extracellular matrix to the cytoskeleton and play important roles in migration, invasion and anoikis resistance. In particular, $\beta1$ integrin is known to confer higher survival and metastatic capacity to a number of cancer cells, including those of prostate origin (180, 181). The serine/threonine protein kinase Akt is a downstream effector of PI3K and an important regulator of various cellular functions, including cell metabolism, transcription, survival and proliferation. Activation of Akt, due to mutations of the phosphatidylinositol 3' kinase (PI3K) p110 catalytic subunit or to loss of the phosphatase and tensin homolog (PTEN) tumor suppressor gene, occurs frequently in human cancers. The cancer cells rely heavily on active Akt to survive after experiencing a number of insults, such as genotoxic stress or growth factor depletion, and to regulate metastasis (182).

The first indication that RAD9 may be related to metastasis came from immunohistochemical analyses of human noncancer and cancer prostate specimens where the protein levels were positively correlated with more advanced stages of the disease (179). Furthermore, a number of *in vitro* metastasis markers such as cell motility, invasion, anoikis resistance and anchorage-independent growth, as well as activation of tumor promoting signaling pathways, specifically integrin expression and Akt activation were examined (183). Suppression of RAD9 protein abundance, by RNA interference, reduced both migration and invasion of DU145 as well as PC3 human prostate cancer cell lines, whereas ectopically expressing *Mrad9*, the mouse homolog of human RAD9, restored the phenotype in these cells (183). Likewise, anchorage-independent growth,

which reflects most faithfully the *in vivo* metastatic potential of a cancer cell, was impaired when *RAD9* was silenced in DU145 prostate cancer cells. Malignant cells have developed mechanisms to evade anoikis and either proliferate without matrix support or enter quiescence until a more suitable environment is presented. Anoikis resistance is, therefore, a prerequisite of tumor metastasis and is considered a hallmark of cancer. Akt kinase plays a pivotal role in the resistance of malignant cells to anoikis. *RAD9* downregulation impaired Akt phosphorylation when prostate cancer DU145 and LNCaP cells were maintained in suspension. Conversely, when *Mrad9* was ectopically expressed in DU145 with reduced levels of endogenous *RAD9*, Akt phosphorylation was restored, and cells became more resistant to anoikis (183). Silencing of *RAD9* leads to a marked down-regulation of integrin β 1. In addition, ectopic expression of *Mrad9* restores integrin β 1 levels when endogenous *RAD9* expression is knocked down in DU145 cells. Furthermore, reduction of integrin β 1 protein levels by a specific siRNA negated the effect of *Mrad9* on migration and invasion, suggesting that *RAD9* affects these metastasis-related processes through the activity of integrin β 1 (183).

In addition to immunochemical data with human prostate specimens and the *in vitro* metastasis assays, gene expression profiling information for human prostate cancer also provides evidence of a role for *RAD9* in tumor progression. Querying publically available datasets (184–186) revealed that the relative *RAD9* mRNA abundance in metastatic prostate tumors is twice as high as in primary prostate tumors (CGB and HBL, unpublished observations).

Given the function of *RAD9* in tumorigenesis, it is reasonable to also consider whether other members of the 9-1-1 complex or *RAD17* can impact the process. ATM/ATR-mediated phosphorylation of human *RAD17*, which recruits 9-1-1 to DNA damage sites, is required for claspin recruitment and *CHK1* activation in response to genotoxic responses (187). However, a phosphorylation-defective *RAD17* can neither recruit the 9-1-1 complex to the damage site, nor induce G_2 checkpoint arrest in response to DNA damage (188). The potential association of *RAD17* with cancer has been demonstrated in a number of studies. It has been shown that *RAD17* acts as a haploinsufficient tumor suppressor that responds to oncogenic stress and loss of *RAD17* is associated with poor prognosis in human B-cell lymphoma patients (189). Likewise, *RAD17* is downregulated in head and neck squamous cell carcinomas (190). In contrast, elevated levels of *RAD17* have been associated with breast (191) and lung carcinomas (192). Furthermore, immunohistochemical analyses have demonstrated that expression of human *RAD17* might correlate with more advanced stages of non-small cell lung carcinoma (NSCLC). Abundance of *RAD17* mRNA was correlated with lymph node metastasis, whereas *RAD17* protein was highly prevalent at the advancing margin of the tumor of

lung cancer tissue but not within the normal lung tissue (193).

Downregulation of *HUS1* and *RAD1* (as well as *RAD9*) lead to defects in DNA replication and cell cycle checkpoint control (194). *HUS1* deficiency, for example, sensitizes mouse embryonic fibroblasts to etoposide-induced apoptosis (195), and *HUS1* downregulation sensitizes human lung carcinoma cells to cisplatin (196). Similarly, *HUS1* or *RAD9* downregulation renders cells susceptible to ionizing radiation (197, 198). However, unlike *RAD9*, there is little information regarding the role of *HUS1* and *RAD1* in tumor initiation and/or progression. In one study, *HUS1* levels correlated significantly with a number of adverse clinicopathologic factors in ovarian cancer, including stage, p53 and *BAX* expression, mitotic index, and apoptotic index (199). On the other hand, heterozygous deletion of mouse *Mrad1* facilitates the development of experimental skin cancer in response to treatment with the carcinogen 7,12-dimethylbenzanthracene (DMBA) (200).

PARP-1

The *PARP-1* protein is an abundant nuclear enzyme that modifies substrates by poly(ADP-ribose)ylation and is involved in the repair of single-strand breaks (116). In response to genotoxic insult, *PARP-1* is recruited to sites of damage, where it becomes activated, and mediates the assembly as well as function of the base excision repair machinery (201). Distinct from its role in DNA repair, *PARP-1* can act as a transcriptional regulator to control a diverse array of functions, including enhancer binding, association with insulators, modulation of chromatin structure, and/or transcription factor regulation (202). *PARP-1* is overexpressed aberrantly in a number of human cancers, including those of breast and prostate (203, 204).

PARP-1 elicits pro-tumorigenic effects in androgen receptor-positive prostate cancer cells, in both the presence and absence of genotoxic drugs. Mechanistically, enzymatically active *PARP-1* plays a critical role in the control of androgen receptor (AR) function. Moreover, in models of advanced prostate cancer, *PARP-1* enzymatic activity is enhanced and regulates castration-resistant AR activity, further linking *PARP-1* to AR activity and disease progression (205).

Recent evidence has uncovered a possible role of *PARP-1* in metastasis. Chromosomal rearrangements involving genes encoding ETS transcription factors (*ERG*, *ETV1*) are found in 50% of human prostate cancer cases (206, 207). Translocations place the coding region of *ERG* or *ETV1* under control of androgen-responsive promoters, such as *TMPRSS2*, thereby activating expression in response to androgens. At least in the case of ETS expressing cancers, the role of *PARP-1* in metastasis appears to be due to its function as a transcription factor, although a role in DNA repair cannot be excluded (205, 208). ETS gene-mediated transcription and cell invasion

require expression and activity of PARP-1 and DNA-PKcs, the kinase involved in NHEJ (204).

PARP-1 is also able to control SNAIL-1 transcription and Snail protein stability (208). Snail is a master regulator of the epithelial-mesenchymal transition (EMT) and has been implicated in key tumor biological processes, such as invasion and metastasis (209). PARP-1 is involved in the activation of SNAIL-1 gene transcription through binding to the integrin-linked kinase (ILK) promoter (210). PARP-1 downregulation has a clear effect on the EMT phenotype, with SNAIL-1 repression and *E-cadherin* upregulation, decreased cell elongation and invasiveness. Furthermore, PARP-1 and NF- κ B together with Snail1 drive expression of the fibronectin gene, which is a typical mesenchymal gene (211). In contrast, PARP-1 has been shown in HaCaT keratinocyte cells to attenuate SMAD-mediated transcription and negatively regulate TGF β -controlled genes involved in the EMT program, such as fibronectin (*FN1*) and N-cadherin (*CDH2*) (212).

BRCA1/2

The breast and ovarian cancer predisposition genes, *BRCA1* and *BRCA2*, encode proteins that are required for efficient homologous recombination repair (213, 214). Germline mutations of *BRCA1* predispose women to breast and ovarian cancers (70). Since its discovery, *BRCA1* has been reported to be involved in multiple functions, all of which control genomic stability in the nucleus, such as cell cycle regulation and checkpoint activation, DNA repair (specifically HR), centrosome regulation, apoptosis and chromatin remodeling (215). *BRCA1* also functions as a transcription regulatory cofactor (216).

Mutations in *BRCA1* do not directly result in tumor formation, but instead cause genomic instability, subjecting cells to a high risk of malignant transformation (217). Furthermore, disruption of *BRCA1* transcriptional activity can be crucial for tumor formation (218). Recent reports have identified novel roles of *BRCA1* in the regulation of caveolin-1 (*CAV-1*) transcription and the inhibition of cell invasiveness (219), as well as the physical association with plasma membrane proteins ezrin-radixin-moesin, thus controlling cell spreading and motility, which have significant implications for tumor invasion and metastasis (220). Interestingly, the *CAV-1* gene is upregulated in cells treated with ionizing radiation and its expression protects cells after exposure through modulating activities of both the HR and NHEJ pathways (221).

BRCA2 is considered a tumor suppressor gene involved in homologous recombination repair of DNA double-stranded breaks (222). In addition to its role in mediating DNA repair and genome stability, *BRCA2* plays a role in the stabilization of stalled DNA replication forks, centrosome duplication, cytokinesis, and transcriptional regulation (214, 223–225). *BRCA2* can suppress tumor development by inhibiting cancer cell growth (226). Loss of *BRCA2* triggers

a proliferative response upon prostate cancer cell interaction with basement membrane proteins (227, 228). Besides its role in cell proliferation, *BRCA2* functions in tumor progression as it can negatively impact on the metastatic potential of prostate cancer cells by down-regulating metalloprotease MMP-9 production through inhibition of PI3K/Akt, thus hindering cancer cell migration and invasion (229).

There is scant clinical information regarding the connection of *BRCA1/2* mutations to the etiology of tumor metastasis. However, it has recently been shown that germline mutations in *BRCA1/2* confer a more aggressive prostate cancer phenotype, with a higher probability of nodal involvement and distant metastasis (230).

NBS1

Nijmegen breakage syndrome (NBS) is a chromosomal instability disorder associated with cancer predisposition, radiosensitivity, microcephaly and growth retardation (231). The gene defective in NBS is *NBS1* (p95, nibrin) and is a member of the DNA double strand break repair complex that also includes *MRE11* and *RAD50* (232). *NBS1* is a putative tumor suppressor gene as shown by the existence of mutations discovered in different tumors (233, 234). *NBS1* is also a prostate cancer susceptibility gene (76). In line with a pro-tumorigenic activity, c-MYC oncogene directly activates *NBS1* expression (235), and *NBS1* overproduction stimulates PI3K activity and enhances cell transformation (236). The induction of tumorigenicity by *NBS1* overexpression may proceed through activation of an oncogenic pathway or the repression of a tumor suppressor, whereas mutations of *NBS1* could also contribute to tumorigenesis through deficiency in DNA repair leading to genomic instability (233, 234, 237).

Evidence that *NBS1* may be associated with metastasis is: (1) *NBS1* overexpression correlates with head and neck squamous cell carcinoma metastasis; (2) *NBS1* overexpression induces EMT through the upregulation of Snail1 levels and matrix metalloprotease, MMP-2 and increases invasiveness/metastasis of head and neck cancer cells as observed both *in vivo* and *in vitro* (238); and (3) *NBS1* upregulates heat shock proteins A4 and A14 with a concomitant increase in the *in vitro* migration, invasion and soft agar colony formation of the lung adenocarcinoma H1299 cell line (239). In addition, it has recently been demonstrated that although *NBS1* haploinsufficiency leads to increased mammary tumor latency in the MMTV-neu mouse model, the tumors that do form are characterized by high metastatic potential (240), further highlighting the role of *NBS1* in tumor metastasis.

RAD51

A critical step in the formation of metastases is cell survival in the bloodstream. Normal and most cancer cells undergo programmed cell death (anoikis) when detached

from their matrices, and metastatic cells must develop specific molecular strategies to survive or even proliferate in an anchorage-independent fashion before they localize to the metastatic site and extravasate. RAD51 is a DNA repair gene involved in tumorigenesis. Its downregulation has been associated with defects in error-free HR DNA repair. However, overexpression of *RAD51*, a rather common occurrence in human cancers (241), contributes to carcinogenesis as it is also associated with aberrant recombination between short repetitive elements and homologous sequences (241). Results with prostate cancer cell lines indicate that anchorage-independence sensitizes cells to genotoxic agents: however it also attenuates a faithful component of DNA repair by targeting the stability of RAD51. This temporal attenuation of HR may contribute to the accumulation of new mutations after DNA damage, which confer a selective advantage to the cells for survival under anchorage independent conditions (242).

XRCC3

The DNA repair protein known as X-ray complementing protein 3 (*XRCC3*), a member of the RAD51 family, participates in HR and is important for the maintenance of chromosome stability as well as DNA damage repair. *XRCC3* affects the invasive behavior of MCF-7 and BT20 human breast cancer cell lines. Specifically, stable or transient overexpression of *XRCC3* increased invasiveness *in vitro* (243). Moreover, *XRCC3* overexpressing MCF-7 cells also showed a high frequency of tumorigenesis *in vivo*, and this phenotype was associated with increased activity of the metalloproteinase MMP-9 and the expression of known modulators of cell-cell adhesion and metastasis, such as CD44 (Receptor for hyaluronic acid), ID-1 (inhibitor of DNA binding 1), DDR1 (discoidin domain receptor tyrosine kinase 1), and TFF1 (trefoil factor 1). These findings suggest a role for *XRCC3* in breast cancer cell line invasiveness and expression of genes associated with cell adhesion and invasion.

ATM

The *ATM* gene product is a serine/threonine protein kinase involved in cell cycle control, DNA repair and chromosomal stability. Its importance to cancer is underscored by the fact that inactivating mutations of *ATM* predispose individuals to the disease Ataxia Telangiectasia (244). Evidence that ATM may promote tumor progression and metastasis has come from recent studies revealing that ATM kinase is hyperactive in late stage breast tumors with lymph node metastasis (245). It was further demonstrated that ATM phosphorylates and stabilizes the epithelial-mesenchymal transition transcription factor SNAIL1, with a concomitant increase in cell migration and invasion *in vitro* and metastasis *in vivo*, which was reversed by inhibiting SNAIL phosphorylation by ATM (245). Likewise, inhibiting ATM kinase activity not only radiosensitizes human

glioma cells, but it also impairs migration and invasion *in vitro*, possibly by reducing Akt and ERK activation (246). The stimulus that maintains high levels of ATM activation in advanced tumors is not known, but hypoxia or oxidative stress, which is able to promote metastasis and ATM activation, could be examples (247–250).

TP53

The p53 protein is involved in DNA damage repair and cell cycle checkpoint control (251). It plays critical roles in maintaining genetic stability, and *TP53* is the most commonly mutated tumor suppressor gene in human cancers (251). Mutations of *TP53* are mainly seen in later tumor stages and coincide with more aggressive types of cancer (252–254). Interestingly, the majority of *TP53* alterations are missense mutations within the DNA binding domain, thus maintaining a full-length protein that has lost its tumor suppressor function (255). However, in addition to losing this function, many mutant p53 proteins also acquire novel, oncogenic activities (256). These gain-of-function p53 mutants lead to increased genomic instability by interfering with proper ATM activation and DNA repair (257, 258). This causes increased incidence of chromosomal translocations (259) and chymothripsis (260), as well as gene amplifications (261). Aneuploidy is promoted by inhibiting assembly of the mitotic spindle checkpoint, as well as inducing centrosome amplification (256). This can inhibit apoptosis by, among other mechanisms, suppressing the function of p73 that induces p53-independent apoptosis after DNA damage (262). p53 inhibits tumor metastasis by multiple mechanisms. For example, p53 controls the transcription of *SMAR1* (scaffold/matrix attachment region binding protein 1), which in turn controls cyclin D1 (*CCND1*) gene expression and inhibits migration as well as invasion by interfering with TGF β signaling in breast cancer (263). Not surprisingly, breast tumors in advanced stages show reduced expression of *SMAR1* (263). In contrast, mutant p53 protein can promote aspects of the metastatic process (264), such as migration and invasion, epithelial to mesenchymal transition, or through the inhibition of p63 protein, which results in increased trafficking of β 1 integrin (265), an integrin that is intimately involved in metastasis in human breast and prostate carcinomas (266–268). The increased rate of recycling of this integrin to the cell plasma membrane correlates positively with cell migration and invasion (269).

GADD45a

The growth arrest and DNA damage gene, *GADD45a*, plays important roles in the control of cell cycle checkpoints, DNA repair (270) and apoptosis (271). Mouse embryonic fibroblasts derived from *GADD45a*-null mice exhibit genomic instability, single oncogene-mediated transformation, loss of normal cellular senescence, increased cellular proliferation, centrosome amplification and

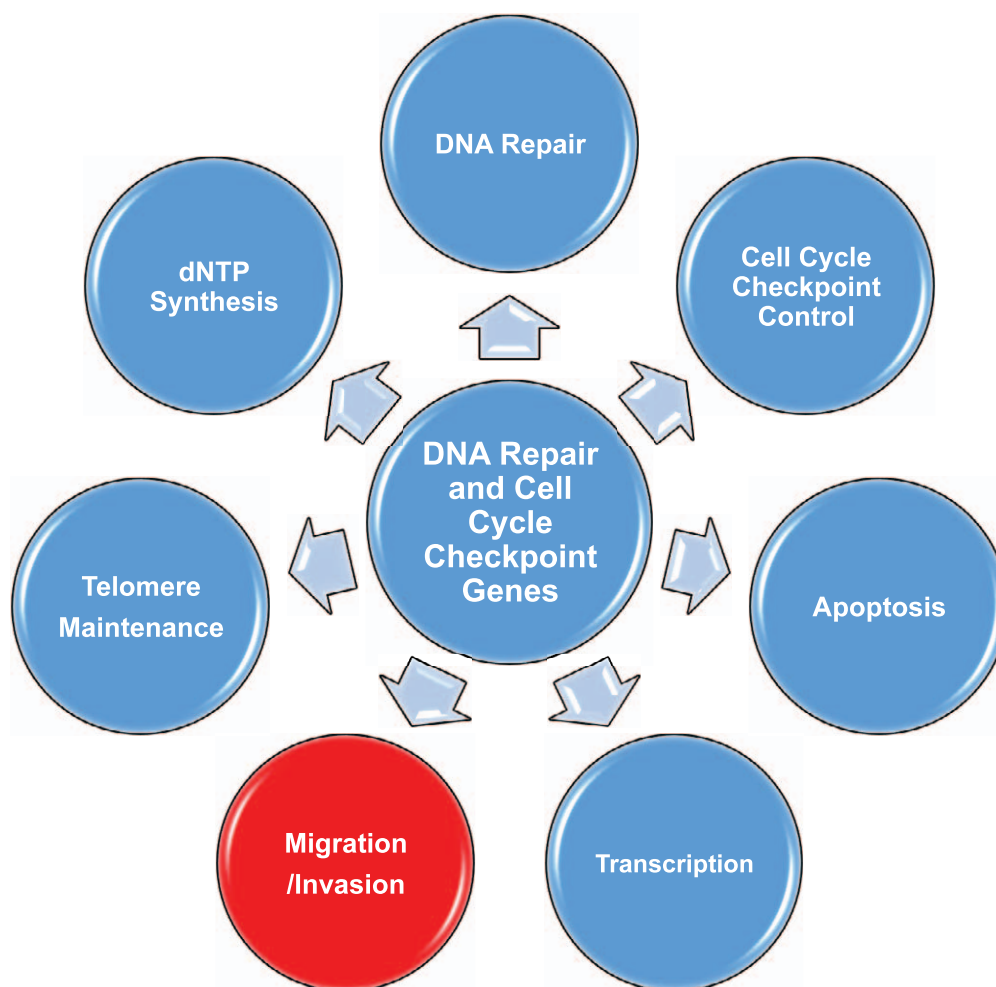


FIG. 1. The multiple functions of DNA damage response and repair genes. Many genes discussed in this review have at least a subset of the activities depicted. For more details see Tables I and II.

reduced DNA repair. A high frequency of *GADD45a* point mutations has been identified in human pancreatic cancer (272), whereas increased gene methylation and decreased protein levels have been shown in breast cancer (273).

A role for *GADD45a* in metastasis is inferred by the observations that it is involved in the control of cell contact inhibition and cell-cell adhesion by enhancing β -catenin protein stability and translocation to the cell membrane (274). Furthermore, *GADD45a* inhibits cell migration and invasion by altering expression of various genes encoding extracellular matrix, cell communication, and cell adhesion proteins (275).

NM23

The gene *NM23* functions in DNA repair and determining whether metastases will form (276). Unlike the other examples where established DNA repair genes are examined for their role in metastasis, *NM23* was first established as an antimetastatic gene, and its significance as a metastasis suppressor has been highlighted in numerous studies (276). Low *NM23* expression in primary melanomas

is correlated with poor clinical outcome, suggesting relevance of *NM23* deficiency to initiation and/or progression in earlier stages of this tumor (277). Subsequent studies revealed that *NM23* participates in DNA repair as well. Importantly, the protein's DNA repair activity is required for its metastasis suppressing function, albeit the exact mechanism remains elusive (278). Recent findings have shown that *NM23*-H1 (isoform H1) participates in nucleotide excision repair (279), however, it is not known whether this function is required for the antimetastatic role.

Other DDR Proteins

A number of other DDR proteins, such as *MCPH1*, 14-3-3 σ , *CDC25A*, *TIP60* and *H2AX*, have been associated with tumor metastasis. Evidence is mainly based on immunohistochemical analyses of clinical specimens or *in vitro* studies.

MCPH1 (microcephalin 1, also known as *BRIT1*), a repressor of human telomerase reverse transcriptase (*hTERT*) function and a key regulator in the DDR pathway, ensures genomic stability and acts as a barrier to the

TABLE I
Functional Roles of DNA Damage Response and Repair Genes

Gene	Role in DDR	Other functions	Refs.
<i>14-3-3σ</i>	Cell cycle checkpoint	—	(283)
<i>ATM</i>	DDR	Cell metabolism	(244, 293)
<i>BRCA1</i>	HRR, cell cycle checkpoint	Centrosome regulation, chromatin remodeling, apoptosis, transcriptional regulation	(213–216)
<i>BRCA2</i>	HRR, cell cycle checkpoint	Centrosome duplication, transcriptional regulation	(214, 222–225)
<i>CDC25A</i>	Cell cycle checkpoint	—	(284)
<i>GADD45a</i>	NER	Transcriptional regulation, apoptosis	(270, 271, 275)
<i>H2AX</i>	DDR, DSBR	—	(290)
<i>MCPH1/BRIT1</i>	HRR	—	(280)
<i>NBS1</i>	DDR	—	(232)
<i>NM23</i>	NER	Nucleoside diphosphate kinase	(276, 279)
<i>PARP-1</i>	BER, HRR	Transcriptional regulation, apoptosis	(116, 201, 202)
<i>RAD9</i>	BER, NER, MMR HRR, TLS, cell cycle checkpoint	Telomere maintenance, dNTP synthesis, apoptosis, transcription factor	(160–166, 172–176, 179)
<i>RAD17</i>	DDR, cell cycle checkpoint	—	(187, 188)
<i>RAD51</i>	HRR	—	(241)
<i>TIP60</i>	DDR, DSBR	—	(285, 286)
<i>TP53</i>	DDR	Transcription factor, apoptosis	(251, 256)
<i>XRCC3</i>	NER	—	(243)

Abbreviations: DDR, DNA damage response; BER, base excision repair; NER, nucleotide excision repair; MMR, mismatch repair; HRR, homologous recombination repair; NHEJ, non-homologous end joining; DSBR, double-strand break repair; TLS, translesion synthesis.

development of cancer (280). MCPH1 level is inversely correlated with the likelihood of breast cancer metastasis (281) or prostate cancer (282). Likewise, inactivation of the G₂/M checkpoint protein 14-3-3σ correlates with lymph node metastasis in nasopharyngeal carcinoma (283). The axis PLK1-CDC25A that permits cells to enter mitosis is often dysregulated in metastatic hepatocellular carcinoma, and cisplatin treatment of metastatic cells does not lead to CDC25A degradation or PLK1 inactivation as normally happens after DNA damage is incurred. As a result, cells enter mitosis, but without mitotic catastrophe, thus leading to increased genetic instability (284). On the other hand, nonmetastatic cells responded to cisplatin with the degradation of CDC25A and the downregulation of PLK1 activity (284).

The tumor suppressor TIP60 is a protein lysine acetyltransferase involved in DNA damage response and repair particularly of double-strand breaks (285), by acetylating and activating, among other substrates, ATM (286). The TIP60 gene is frequently downregulated in colon and lung carcinomas (287). Moreover, downregulation of TIP60 correlates with distant metastasis in colon cancer (288), as well as melanoma (289). In the latter case, ectopic expression of Tip60 in melanoma cells reduced and knockdown increased *in vitro* cell migration, pointing further to a potential role of Tip60 in metastasis (289).

Histone H2AX is an important effector of the DNA damage response that is responsible for recruiting cell cycle checkpoint and DNA repair factors to sites of double-strand breaks (290). By facilitating the DNA damage response and repair, H2AX functions as a tumor suppressor. *H2AX* maps to chromosome 11q23, a region that is deleted or mutated in

a variety of human malignancies, including leukemia, breast and head and neck cancers (290). However, H2AX can also promote tumor growth and pathologic angiogenesis under conditions of hypoxia (291) and therefore aid the dissemination of tumor cells to metastasize to distant sites. H2AX is needed for endothelial cell proliferation under hypoxic conditions and for hypoxia-driven neovascularization, whereas genetic ablation of *H2AX* reduces the proliferation of these cells *in vitro* and *in vivo* (291). However, it is still not clear whether the DNA repair function of H2AX is required for the regulation of endothelial cell proliferation under hypoxia conditions (292).

CONCLUSIONS

In recent years, it has become apparent that many genes classically thought to operate in DNA repair or checkpoint control also have roles in carcinogenesis and in particular tumor metastasis as well. However, the molecular mechanisms involved are not completely understood. Most published experiments are correlative in nature, and therefore, at present, cause-effect relationships between most of these genes and cancer metastasis cannot be unambiguously assigned. The evidence that DNA repair or checkpoint genes participate in metastasis comes mainly from genome profiling data of primary versus metastatic tumors, or from immunohistochemical analyses of patient tumor specimens. In addition, *in vitro* data have linked expression of some of these genes to increased migration and invasion, anoikis resistance and anchorage-independent growth. Molecularly, DNA repair or cell cycle checkpoint genes affect pro-tumorigenic and pro-metastatic pathways

TABLE II
Role of DNA Damage Response and Repair Genes in Tumor Growth and Metastasis

Gene	Tumor growth	Metastasis	Refs.
<i>14-3-3σ</i>	Tumor suppressor	Inactivation associates with metastasis	(283)
<i>ATM</i>	Tumor promoter	SNAIL1 phosphorylation and stabilization, correlates with metastasis	(245–250)
<i>BRCA1</i>	Tumor suppressor	Metastasis suppressor, regulation of cav-1	(70, 217–220, 230)
<i>BRCA2</i>	Tumor suppressor	Downregulation associates with metastasis	(226–230)
<i>CDC25A</i>	Tumor promoter	Persistence of CDC25A expression associates with metastasis	(284)
<i>GADD45a</i>	Tumor suppressor	Migration, invasion inhibition, β-catenin exclusion from nucleus, correlates with decreased metastasis	(272–275)
<i>H2AX</i>	Tumor suppressor	Angiogenesis, correlates with metastasis	(290, 291)
<i>MCPH1/BRIT1</i>	Tumor suppressor	Expression is inversely correlated with metastasis	(281, 282)
<i>NBS1</i>	Tumor suppressor in some cancers, tumor promoter in others	SNAIL1, MMP-2 upregulation, EMT, migration, invasion, soft agar colony formation, elevated <i>in vivo</i> metastasis	(76, 231, 233–240)
<i>NM23</i>	No effect	Metastasis suppressor	(276–278)
<i>PARP-1</i>	Primary tumor growth	SNAIL1 transcription, EMT, invasion; may associate with metastasis	(203–205, 208)
<i>RAD9</i>	Tumor promoter in some cancers, tumor suppressive activity in others	Migration, invasion, anoikis resistance, anchorage-independent growth, ITGB1 expression control, increased levels correlate with metastasis	(160, 179, 183)
<i>RAD17</i>	Tumor promoter in some cancers, tumor suppressor in others	Overexpression correlates with metastasis	(189–193)
<i>RAD51</i>	Tumor promoter	Destabilized under anchorage-independent conditions that may increase mutations and favor metastasis	(241, 242)
<i>TIP6</i>	Tumor suppressor	Downregulation correlates with metastasis	(287–289)
<i>TP53</i>	Tumor suppressor	Metastasis inhibitor, mutant p53 promotes metastasis by modulating ITGB1 stability	(251–254, 263–265)
<i>XRCC3</i>	Tumor promoter	Invasion, MMP-9, CD44, ID-1, DDR1, TFF1 expression, associates with metastasis	(243)

Notes. A direct link between protein/mRNA expression levels and/or gene mutations and metastasis has been demonstrated only for NBS1 and NM23, whereas in all other cases there is only an indirect correlation with metastasis, based either on gene expression microarray or immunohistochemical analyses of human cancer specimens or *in vitro* biological characteristics that may predict *in vivo* metastasis behavior. Abbreviations: EMT, epithelial to mesenchymal transition; MMP, matrix metalloprotease; ITGB1, integrin β1; CD44, CD44 antigen; ID-1, inhibitor of differentiation-1; DDR1, discoidin domain receptor tyrosine kinase 1; TFF1, trefoil factor 1; cav-1, caveolin-1.

involving Akt activation, integrin expression, as well as transcriptional control of genes involved in epithelial-to-mesenchymal transition and metalloproteases. It is therefore necessary to prove that the encoded proteins drive metastasis in animal models to demonstrate directly a cause-effect relationship. So far, this has been shown only for NBS1 in head and neck cancer cell lines by an *in vivo* tail vein metastasis assay (238).

Proteins that participate in DNA repair or cell cycle checkpoint control usually have multiple cellular, biochemical or molecular functions, as shown schematically in Fig. 1 and detailed in Tables I and II. An emerging common theme for many DNA repair proteins is that they can function in gene regulation as transcriptional factors or cofactors. RAD9, for example, can function as a sequence specific transcription factor and regulate a number of genes including *p21^{waf1/Cip1}* (177), whereas BRCA1, which lacks sequence specific DNA binding, can be recruited to

promoters by sequence specific transcription factors and act as a transcriptional co-activator or co-repressor (216). Likewise, GADD45a alters global transcript abundance, affecting proteins important for cell migration and invasion, such as those impacting on cell–cell adhesion (275).

Which of the multiple functions of DNA repair or cell cycle checkpoint control proteins is actually required for regulating metastases is not clear. One can speculate that DNA repair activity is required. On the other hand, the transcriptional regulatory activity of numerous DNA repair proteins will certainly be important. It is already known that DNA repair proteins control, at the transcriptional level, the abundance of many other proteins involved in metastatic pathways and certainly more will be discovered as studies progress. Finally, specific protein–protein interactions of DNA repair or checkpoint factors with as yet unidentified proteins will also likely influence metastasis. A very complex picture is emerging from all of these investiga-

tions. Nevertheless, from a pragmatic perspective, resolution of the molecular mechanisms involved is important to facilitate the design of therapies that target DNA repair and cell cycle checkpoint proteins as novel anti-cancer agents.

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