Review

Molecular Cancer Research

Transcriptional Roles of PARP1 in Cancer

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Abstract

Poly (ADP-ribose) polymerase-1 (PARP1) is an abundant, ubiquitously expressed NAD^+-dependent nuclear enzyme that has prognostic value for a multitude of human cancers. PARP1 activity serves to poly (ADP-ribose)-ylate the vast majority of known client proteins and affects a number of cellular and biologic outcomes, by mediating the DNA damage response (DDR), base-excision repair (BER), and DNA strand break (DSB) pathways. PARP1 is also critically important for the maintenance of genomic integrity, as well as chromatin dynamics and transcriptional regulation. Evidence also indicates that PARP-directed therapeutics are "synthetic lethal" in BRCA1/2-deficient model systems. Strikingly, recent studies have unearthed exciting new transcriptional-regulatory roles for PARP1, which has profound implications for human malignancies and will be reviewed herein. *Mol Cancer Res; 12(8); 1069–80. ©2014 AACR.*

Introduction

Poly (ADP-ribose) polymerase-1 (PARP1) is an enzyme responsible for approximately 90% of the ADP-ribosyl transferase activity [poly (ADP-ribose)ylation (PARylation)] in both nontransformed and malignant human cells (1), the majority of which is self-directed (1, 2). The PARP family of enzymes contains 18 family members, PARP1 being the first to be characterized (3), which PARylate client proteins using NAD^+ as a cofactor, and thereby control a diverse set of biologic functions (4). The first defined role for PARP1 was to orchestrate DNA damage resolution, especially in the context of base excision repair (BER; ref. 5). However, subsequent studies implicated PARP1 as harboring pleiotropic cellular functions, including DNA repair/maintenance of genomic integrity, DNA methylation, regulation of circadian clocks, chromatin regulation, and histone modification (2, 6–8). *Parp-1*-deficient mouse models are viable and demonstrate increased sensitivity to genotoxic stress, resistance to DNA damage–induced cell death, increased tumorigenesis in chemically or genetically induced models (reviewed in ref. 2) and altered hypoxic response (9). Cell models of *Parp1* deficiency demonstrate altered transcription of p53 targets (ref. 10; heat shock factor 1; ref. 11). Most recently, means by which PARP1 regulates gene transcription have been identified (2, 6, 7, 12); the present review addresses the function and consequence of PARP1-regulated transcription in the context of human malignancies.

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Regulation of PARylation

PARP1 is a DNA-dependent ADP-ribosyl transferase that is localized in the nucleus and is frequently associated with chromatin (1, 2, 12). The capacity of PARP1 to associate with DNA is manifested via direct binding and/or interacting with nucleosomes and other chromatin-associated proteins, including transcription factors (13), the transcriptional machinery (14, 15), and chromatin modifiers (1, 2, 12). The enzymatic activity of PARP1 is regulated by what it is bound to, such as damaged DNA or other nuclear proteins (1, 12, 16–21), as well as posttranslational modifications, such as autoPARylation (inhibitory; refs. 22–26) and phosphorylation by ERK1/2 (activating, DNA independent; refs. 27, 28). In addition, an NAD^+ synthase [nicotinamide mononucleotide adenylyltransferase-1 (NMNAT-1)] associates with PARP1, thus allowing for a proximal source of NAD^+ cofactor and increasing PARP1 activity (ref. 29; Fig. 1A).

The first identified roles of PARP1 were associated with DNA damage and genomic maintenance, with specific roles in BER, single-strand (SSB), and double-strand break (DSB) repair (5). In response to DNA damage, PARP1 enzymatic function is activated, and persists in a correlative manner with the extent of the damage. When DNA breaks are repairable, PARP1 regulates repair and cell survival; but in response to catastrophic damage, PARP1 regulates the induction of cell death (30–32). In addition to playing roles in SSB and DSB repair (33–35), PARP1 has been implicated in homologous recombination (HR) at stalled or collapsed replication forks (36, 37), as well as regulating nonhomologous end-joining (NHEJ; refs. 38–41). Whereas the PARP family of enzymes is responsible for PAR anabolism, the PAR glycohydrolases (PARG) regulate PAR catabolism. PAR hydrolysis is regulated by a number of isoforms of the single *PARG* gene that arise from splicing. The long isoforms of PARG shuttle between the cytosol and the nucleus, whereas the short isoform is exclusively cytoplasmic (42–45).

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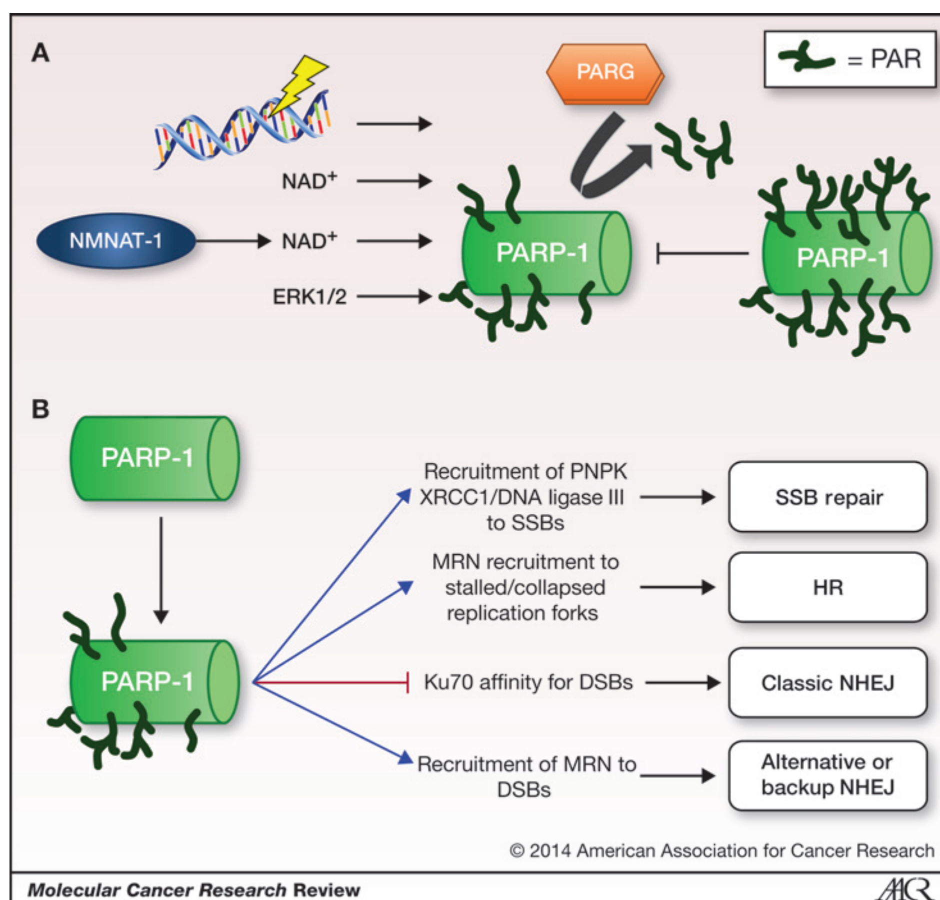

Figure 1. A, Poly (ADP-ribose) polymerase-1 (PARP1) enzymatic activity is activated by multiple stimuli, including: DNA damage, nicotinamide adenine dinucleotide (NAD+), proximal NAD+ produced by Nicotinamide Nucleotide Adenylyltransferase 1 (NMNAT1), which interacts with PARP1, and phosphoryltion by ERK1/2. The majority of PARP1 enzymatic activity is self-directed, as depicted by the Poly (ADP-ribose) chains (PAR), and hyper-PARylated PARP1 inhibits PARP1 enzymatic activity, as depicted by the squared line. PAR moities are cleaved by Poly (ADP-ribose) glycohydrase (PARG) members. B, Enzymatically active PARP1 stimulates (blue arrows) or inhibits (blocked red line) the recuritment and downstream DNA damage repair functions of multiple DNA repair factors as depicted. SSB, single strand break. HR, homologous recombination. NHEJ, Non-homologous end joining.

Although the PARP superfamily and PARG play clearly important and distinct roles in PAR metabolism, the role of PARG/PARP1 interplay in cancer remains poorly described. In addition, PAR catalysis in the mitochondria has been discovered to be performed by ADP-ribosylhydrolase 3 (ARH3), not PARG, for which very little is known in the context of cancer (46–48).

Current understanding of PARP1-driven PARylation is that in response to stimuli (such as DNA damage; Fig. 1B), PARP1 enzymatic function is robustly and rapidly induced, using NAD^+ as the donor for ADP-ribose resulting in multiple biologic outcomes, and PAR is degraded by PARG to ADP-ribose monomers. These terminal ADP-ribose monomers are then removed by the recently discovered TARG1, whose deficiency results in neurodegenerative disease (49). PAR moieties generated can noncovalently interact with multiple domains that result in controlling of multiple pathways (reviewed in refs. 46, 50). In addition, recent proteomics work has begun to define the PARylated proteome upon various stimuli and how these PARylated proteins are often involved in chromatin organization and transcriptional regulation, as well as the DNA damage response (DDR; ref. 51–53). Here, the major transcriptional regulatory functions of PARP1, and the downstream biologic consequence(s) of these events for human malignancies are discussed. Specific areas of focus address (i) modulation of tumor suppressor and oncogene function, (ii) regulation of effectors of the metastatic process, (iii) regulation of cell survival and adaptation, and (iv) transcriptional regulation in hormone-dependent cancers.

Basic Mechanisms of PARP1-Mediated Transcriptional Control

Although initially characterized as a factor intimately involved in regulation of DNA repair/genomic maintenance, PARP1 was recently demonstrated to exert pleiotropic roles in transcriptional regulation in cancer and noncancer model systems (2, 6, 7, 12). Under NAD^+-depleted conditions, PARP1 compacts chromatin by binding to and bringing together nucleosomes and can also affect chromatin structure through PARylation of histones, thus disrupting nucleosomes and resultant chromatin architecture (1, 24, 54–56). PARP1 has also been found to localize to promoters of actively transcribed genes and prevent binding of Histone H1, thus promoting transcriptionally active chromatin (57). The transcriptional regulatory functions of PARP1 are multi-fold, do not universally require enzymatic activity, and are manifest through divergent functions, including enhancer binding, association with insulators, modulation of chromatin structure, and/or direct transcription factor regulation (as either a context-dependent

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coactivator or corepressor). PARP1 binding at regulatory loci of genes does not always correlate with activation of transcription. In fact, PARP1 binding can sometimes correlate with transcriptional repression (2, 6, 7, 12). As such, transcriptional regulation by PARP1 can be either positive or negative, occur through multiple mechanisms, and is complex and cell- and context-specific (Fig. 2A). The cancer context–dependent mechanisms by which PARP1 modulates transcription is discussed below (Fig. 2B), particularly as related to chromatin remodeling, modulation of tumor suppressor and oncogene function, transcriptional regulation of the metastatic process, modifying cell survival and adaptation, and nuclear receptors (NR) in hormone-dependent cancers.

PARP1 regulates select ATPases that control chromatin remodeling, such as amplified in liver cancer 1 (*ALC1*), an ATPase in the SNF2 superfamily that is frequently deregulated in hepatocellular carcinoma. *ALC1* demonstrates sequence similarity to other chromatin remodelers (such as SNF2, ISWI, and CHD1), but lacks any domains with known function in chromatin architecture regulation. However, *ALC1* has a macrodomain, which has been demonstrated to serve as a binding domain for PAR (58). Moreover, ALC1 was found to have ATPase activity dependent upon both PARP1 and NAD^+. This ATPase function was correlated with ALC1 binding to and remodeling of chromatin, likely due to activation by PAR moieties involved in PARP1 automodification (58). Although there is no explicit link to transcription, this study demonstrates that PARP1 activates the ATPase capacity of ALC1, which is frequently deregulated in human hepatocellular carcinoma. Further mechanistic insight was gained upon the discovery of and ALC1–PARP1 nucleosome intermediate that was stable and required for activation of ALC1 activation and chromatin remodeling (59). Additional research has demonstrated a critical connection between PARP1 and ALC1 in regulating chromatin remodeling in the context of the DDR (60–62), serving as a model of the dual roles of PARP1 in regulating DNA damage and chromatin structure, even in the context of the same partnering molecule.

Distinct from these roles, PARP1 controls the function of selected insulators that modulate chromatin architecture in models of cancer. For example, CTCF (CCCTC-binding factor) is a transcription factor that performs a multitude of transcriptional-regulatory roles dependent on its posttranslational modification status and interactions with other molecules. PARylation is requisite for the ability of CTCF to serve as an insulator (blocking interaction between regulatory loci) and a barrier (inhibiting the spread of heterochromatin). In addition, it was shown that CTCF activates PARP1, resulting in DNA hypomethylation, which has been linked to cancer initiation and progression. Interestingly, comparison of existing datasets of genome-wide CTCF and PARP1 residence on chromatin revealed that sites of overlap (deemed "hot spots") are both intergenic as well as intragenic, and varied between chromosomes. Because these hot spots were clustered within the genome (63), it was suggested that this might be linked to specificity of PARP1 and CTCF at these loci. These data correlate PARP1 to the insulating capacity of CTCF.

In addition to chromatin-remodeling factors and insulators, PARP1 can impinge upon nucleosome accessibility. Seminal work in understanding the relationship between PARP1 and transcription showed that PARP1 and histone H1 compete for binding to nucleosomes and have an inverse binding pattern at actively transcribed genes (57). Further delineation of the mechanisms by which PARP1 positively controls gene transcription revealed that PARP1 promotes the binding of RNA polymerase II (RNApolII) and associated transcriptional machinery to actively transcribed genes in MCF-7 breast cancer cells. This increase in residence correlated with increased chromatin accessibility at the transcriptional start sites of these genes and trimethylation of histone H3 lysine 4 (H3K4me3), a hallmark of active transcription. Further mechanistic insight led to the discovery that PARP1 blocks the ability of the KDM5B demethylase to demethylate H3K4, thus promoting transcription of these PARP1-regulated genes. It was determined that demethylation of H3K4 allows for histone H1-driven expulsion of RNA polymerase II from active promoters, and that PARP1-dependent inhibition of KDM5B-mediated H3K4 demethylation permits a transcriptionally competent chromatin environment. PARP1 enzymatic activity was determined to be required for limiting KDM5B binding to active promoters, and KDM5B was shown to be a target of PARylation; thus, deregulation of PARP1 enzymatic activity could lead to inhibition of KDM5B demethylase activity (64). These findings provide an elegant delineation of the means by which PARP1 regulates transcription, and exemplifies how the transcriptional regulatory capacity of PARP1 can be assessed.

Although PARP1 is responsible for the majority of PARylation, the role of PARG must also be considered. On the basis of the opposing functions of PARP1 and PARG with respect to PARylation, it might be predicted that transcription is disparately regulated by PARP1 and PARG. Genome-wide analyses using isogenic models of PARP1 or PARG depletion in breast cancer cells revealed an approximate 50% overlap in genes regulated by PARP1 ($\sim$1,200 genes) and PARG ($\sim$1,100 genes; both positive and negative regulation), which was typically in the same direction (up or down) and of similar magnitudes. This intersect of approximately 500 genes was enriched for ontologies associated with metabolism and the stress response, suggesting that PARP1 and PARG may separately or coordinately regulate these processes. Further examination of the PARP1- and PARG-regulated transcriptome revealed that PARP1 and PARG localize to the promoters of both upregulated and downregulated genes, and binding of both were proportional in all the genes examined. Additional studies demonstrated that both PARP1 and PARG can regulate the chromatin occupancy of the other, and there was gene context–specific dependency on the enzymatic activity of PARP1 and PARG in transcriptional regulation. Together, these studies indicate that despite the opposing functions of PARP1 and PARG (catabolism vs. anabolism of PAR), they bind to

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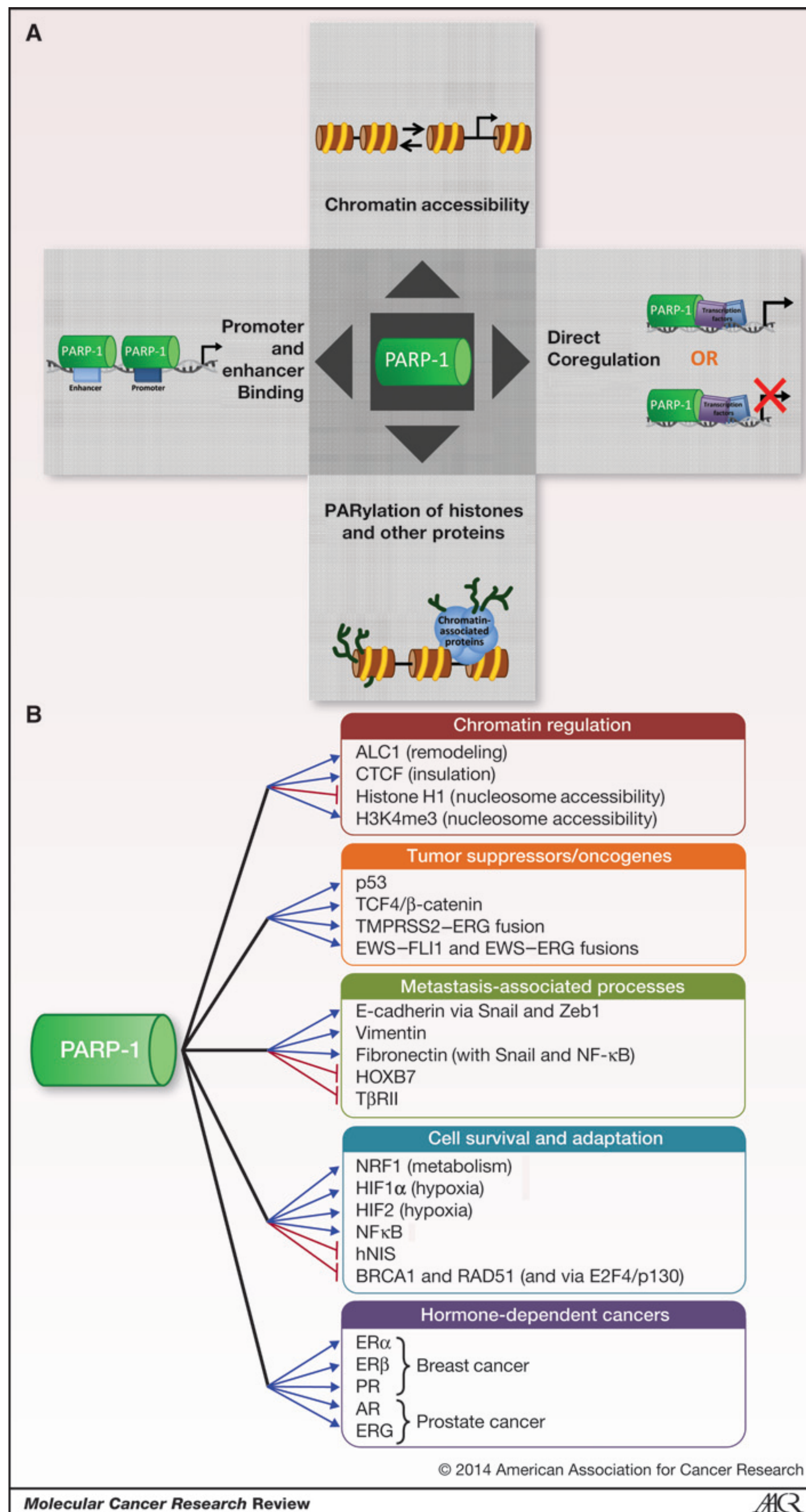

Figure 2. A, PARP1 regulates transcription via distinct and non-mutually exclusive mechanisms including: altering chromatin accessibility (top), serving as a direct co-regulator of transcrition factor function (right), PARylating histones and other chromatin associated proteins (bottom), and binding to and functioning at gene regulatory loci such as promoters and enhancers (left). B, PARP1 has been shown to regulate the function of many chromatin-associated proteins and transcription factors. Activating regulation is depicted by blue arrows, inhibiting regulation is depicted by red blocked lines.

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target promoters and tend to act in a similar fashion in their capacity to regulate transcription (65).

Taken together, it is clear that there are both context-specific and general mechanisms and consequences of PARP1-regulated transcription and chromatin remodeling in human malignancy. Although the functions of PARP1 in regulating a specific set of transcription factors and chromatin modulators (ALC1, CTCF, and KDM5B) have been outlined above, whether these observations hold true for other transcriptional-regulatory processes remains to be assessed, and would lead to not only greater mechanistic understanding of PARP1-dependent transcriptional regulation, but could uncover new therapeutic opportunities in the context of cancer management.

Modulation of Tumor Suppressor and Oncogene Function by PARP1

Complementing the transcriptional and chromatin-regulatory functions of PARP1 (described above), PARP1 also directly modulates sequence-specific transcription factors, including several of high relevance for human malignancies. Notably, PARP1 is in transcriptional-repressive complexes with p53, and PARylation of p53 within in this context results in recruitment of HDAC1 and HDAC2. This transcriptional-repressor complex blocks the expression of metastasis-associated protein 1 (MTA1), which is involved in nucleosome remodeling and transcriptional repression, is frequently enriched in a number of cancers, and is associated with disease progression and metastasis. Abrogation of PARP1-dependent MTA1 repression results in elevated levels of hypoxia-inducible factor (HIF)1α and VEGF, suggesting that PARP1-mediated p53 transcriptional function negatively regulates MTA1 expression and cancer-associated genes and phenotypes (66). As such, in addition to maintaining genomic integrity, part of the tumor-suppressive roles for both p53 and PARP1 may include regulation of MTA1. Other studies have implicated a functional interaction between PARP1 and p53 in multiple biologic functions (reviewed in refs. 2, 67). Specifically, it has been identified that PARP1 regulates the p53-mediated DDR via stabilization of p53 in response to radiation (10), and PARP inhibition suppresses the activation of p53 in response to radiation (68). PARP1 activation results in ATP depletion and subsequently reduced TAF1 kinase activity and p21 activation (69). *Parp1*–null mouse embryonic fibroblasts (MEF) have approximately 2× lower basal p53 expression and DNA damage–dependent reduction than wild-type MEFs (70), and functional PARP1 is required for p53-dependent cytotoxicity in response to proteasome inhibitors (71).

In addition to p53, PARP1 regulates organ site–specific tumor suppressors. For example, loss of function of the *APC* (adenomatous polyposis coli) tumor-suppressor gene is frequently associated with familial and sporadic colorectal cancer, resulting in accumulation of β-catenin and activation of T-cell factor (TCF)/lymphoid enhancer factor (LEF) transcription factors. PARP1 interacts with TCF4, and in complex with TCF4/β-catenin in colorectal cancer; through this function, PARP1 increases transcriptional activation of TCF/LEF by β-catenin (72). Conversely, Ku70 has also been observed to associate with TCF4/β-catenin and repress TCF/LEF function, and Ku70 competes with PARP1 for binding to the complex. Consonantly, PARP1 mRNA and protein levels are elevated in familial adenomatous polyposis (FAP) and sporadic colorectal cancer clinical specimens, suggesting a possible causative role of PARP1-regulated transcriptional activation of TCF/LEF in colorectal cancer. Furthermore, it was observed that Ku70 mRNA was decreased in four of five cases of sporadic colorectal cancer compared with matched normal tissue, suggesting that PARP1-mediated TCF/LEF activation may be increased in human disease. Finally, increased nuclear PAR is observed in colorectal adenoma clinical specimens as compared with matched normal tissue (73). Together, these studies demonstrate that PARP1 can positively regulate the transcriptional activity of TCF/LEF in the context of colorectal cancer, and that PARP1 is both expressed to a higher degree and is more active in colorectal cancer (72, 73), thus suggesting that PARP1 transcriptional-regulatory function may be worthy of further examination as a therapeutic target in future management of colorectal cancer.

As described above, PARP1 regulates the function of classic tumor-suppressor genes (*p53* and *APC*), but there are many roads to tumorigenesis, and consequently many cancers overexpress oncogenic E-twenty-six (ETS) transcription factors via gene fusions. In prostate cancer, gene fusions occur between the *TMPRSS2* and *ERG* (an ETS transcription factor) genes in more than 50% of prostate cancer. As TMPRSS2 is a well-described androgen receptor (AR) target gene, this places *ERG* expression under control of AR, which plays a critical role in prostate cancer. Generation of these fusions seems to be an early event in prostate tumorigenesis, and the *TMPRSS2–ERG* gene fusion has been found to induce cancer cell growth and invasion. Recent studies revealed that the *TMPRSS2–ERG* fusion gene product interacts with PARP1, and that PARP1 expression is required for the transcriptional activation function of ERG. PARP1-dependent ERG activity was found to drive invasive phenotypes, and PARP inhibition diminished ERG-driven cell invasion, intravasation, and metastasis, as well as xenograft tumor growth in fusion-positive tumor cells. Interestingly, ETS function was shown to promote DNA damage, and this could be exacerbated by PARP inhibition, suggesting that in the context of *TMPRSS2–ERG*–positive prostate cancer (74), PARP1 serves in both DDR as well as the transcriptional regulation of oncogenic fusion protein function. As such, targeting both of these functions with PARP inhibitors may serve therapeutic benefit in prostate cancer.

ETS fusions are not solely found in prostate cancer, as Ewing sarcomas are tumors of bone and soft tissue that are characterized by chromosomal translocations that fuse a portion of Ewing's sarcoma breakpoint gene 1 (*EWS*; responsible for transcriptional activation) to the DNA binding domain of either *FLI1* or *ERG*. The resulting translocation-induced chimeric proteins regulate cell proliferation,

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invasion, and tumorigenesis; thus, development of means to target these proteins therapeutically could provide clinical benefit. Notably, both the EWS–FLI1 and EWS–ERG fusion gene products interact with and depend on PARP1 function. Expression of either fusion product enhanced DNA damage and increased their invasive potential (events that were mitigated by PARP inhibition). In addition, PARP inhibition was found to reduce EWS–FLI1–dependent tumor growth and metastasis *in vivo*. Finally, knockdown of either PARP1 or DNA-PK resulted in diminished EWS–FLI1 target gene expression in a similar fashion as *EWS* knockdown, demonstrating that PARP1 potentially plays a key role in EWS–FLI1 fusion protein transcriptional function. It was proposed that PARP1 and EWS–FLI1 function in a feed-forward mechanism in Ewing sarcoma, whereby the fusion protein directly induces PARP1 mRNA and protein expression, culminating in increased EWS–FLI1 transcriptional activity (75). This study provides an example of the capacity of PARP1 to regulate both DDR as well as transcription, ultimately demonstrating that targeting both facets of PARP1 function pharmacologically may significantly alter cancer therapy.

Although limited in number, these studies collectively suggest that PARP1 modulates the transcriptional function of both tumor suppressors and oncogenes, exemplifying the capacity of PARP1 to elicit context-specific pro- or antitumor effects. Further understanding of the underlying mechanisms of PARP1 function will thereby be of utmost importance for refined utilization of PARP inhibitors in cancer therapy.

Regulation of Effectors of the Metastatic Process by PARP1

Intriguingly, the transcriptional regulatory function of PARP1 has also been implicated in epithelial-to-mesenchymal transition (EMT), a cellular process thought to promote metastatic events. Loss of E-cadherin expression is believed to represent a key event in EMT, resulting in disorganization of cell-to-cell junctions. Although multiple factors control E-cadherin expression (including promoter methylation, mutation, and dysregulated transcription), the transcription factors Snail and Zeb1 are key effectors of E-cadherin expression. In brief, Snail and Zeb1 are upregulated in a subset of malignancies and suppress E-cadherin expression. PARP1 negatively controls cancer-associated Snail and Zeb1 expression resulting in E-cadherin expression, thus providing some of the first evidence that PARP1 may serve to inhibit EMT through its role in transcriptional regulation (76). In addition to regulating E-cadherin expression through Snail, PARP1 also collaborates with Snail and NF-κB to drive expression of another EMT factor, fibronectin (77). Although the functional interaction of Snail, NF-κB, and PARP1 in the context of fibronectin regulation has been delineated in a small selection of cancer models, the biologic consequence(s) and contribution to EMT are yet to be explored.

Along with loss of E-cadherin, vimentin expression is also associated with EMT. Consistent with a role in EMT regulation, PARP1 binds to and directly regulates transcription from the vimentin promoter. Interestingly, this transcriptional activation was independent of the enzymatic activity of PARP1, and could be suppressed by H_2O_2, which is an activator of PARP1 enzymatic function. H_2O_2 treatment resulted in diminished PARP1 protein expression as well as vimentin protein expression, and induced overexpression of PARP1 resulted in greater H_2O_2-induced repression of vimentin promoter activity, suggesting that PARP1 may play an active role in the inhibition of vimentin expression (78). Although the study described here clearly implicates PARP1 in the transcriptional regulation of vimentin, further study about the biologic consequence is needed.

Further evidence that PARP1 transcriptionally controls EMT is by regulation of HOXB7, which is overexpressed in some instances of breast and ovarian cancer and is capable of inducing EMT. HOXB7 associates with PARP1 in models of breast cancer (SKBR3), and HOXB7 is also a target of PARylation. PARylation of HOXB7 by PARP1 reduces HOXB7 affinity for DNA and subsequently the transcriptional activity of HOXB7. Thus, at least in the context of one HOX family member (HOXB7), PARP1 serves to negatively regulate HOX transcriptional activity (79). Finally, the means by which PARP1 regulates EMT may be due, in part, to regulation of the transforming growth factor β (TGFβ) signaling, which is capable of inducing EMT. In nontransformed cells, TGFβ inhibits cell proliferation and induces differentiation. In certain contexts, tumor cells bypass TGFβ signaling by deregulation of the receptor for TGFβ, TGFβ receptor type II, whose expression is negatively regulated by PARP1 (80).

Combined, it is clear that PARP1 plays a role in the transcriptional regulation of events associated with EMT by regulating the expression (E-cadherin, fibronectin, and vimentin) or function (HOXB7 and TGFβ) of key players in the process of EMT. However, it is not clear whether the net effect of PARP1-regulated transcription is to drive or block EMT, and may be contingent on the type of malignancy studied. As such, this complicated network merits further examination in the context of human malignancy.

Regulation of Cell Survival and Adaptation

Intriguingly, the transcriptional-regulatory function of PARP1 has also been implicated in cell survival and adaptation processes, largely associated with metabolism, hypoxia, and DDR.

With regard to metabolism, nuclear respiratory factor 1 (NRF1) is a transcription factor involved in the regulation of mitochondrial biogenesis, translation/protein stability, DNA synthesis, DDR, and proliferation. NRF1 can interact with DNA and PARP1 simultaneously, and PARP1 PARylates NRF1, thus causing decreased interaction between PARP1 and NRF1. However, PARP1 expression was found to be required for optimal transcriptional activation of NRF1 target genes (81), suggesting that PARP1 plays a key role in the transcriptional regulation of cellular metabolism.

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A second adaptive process that PARP1 is thought to influence is the hypoxic response. The hypoxic response is regulated transcriptionally by HIF subunits α and β, the α subunits being continuously transcribed and translated and regulated by the von Hippel-Lindau (VHL) tumor suppressor in an oxygen availability–specific manner. In an initiation/promotion model of skin carcinogenesis, PARP1 inhibition delayed tumor promotion, and is associated with decreased inflammatory infiltration, reduced mitosis, diminished apoptosis (in noncancerous tissue, but not in cancerous tissue), and decreased tumor vasculature. These phenotypes correlated with diminished AP-1 DNA binding, but not NF-κB DNA binding, as well as decreased HIF1α mRNA and protein expression. Consequently, HIF1-dependent gene regulation of the hypoxic-responsive transcriptional network was severely compromised (82). In a model of chronic myelogenous leukemia (CML), it was found that PARP1 and HIF1α interact and cooperate to activate HIF target gene expression. This was dependent upon PARP1 enzymatic activity, but did not result in altered HIF1α stability or DNA-binding capacity. PARP1 knockdown caused diminished HIF1α target gene expression, which correlated with increased necrotic tumor cell death and diminished tumor vascularization, but paradoxically no change in CML tumor growth (83). Thus, PARP1 functions to positively regulate HIF1α in multiple models of oncogenesis. Although HIF2α has approximately 50% sequence identity to HIF1α and there is some overlap in the transcriptional programs regulated by HIF1 and HIF2, some targets are differentially regulated. In a cell model system of renal cell carcinoma lacking VHL, it was found that HIF2 and PARP1 form a complex under hypoxic conditions dependent upon PARP1 enzymatic activity. Further examination in other model systems indicated that depletion of PARP1 protein results in diminished HIF2α mRNA expression, reduced hypoxia-induced HIF2α protein expression, and subsequent HIF2α target gene expression (9). In sum, PARP1 seems to be a positive regulator of both HIF1 and HIF2 expression and transcriptional function, and the subsequent hypoxic response.

With regard to DDR, NF-κB represents a group of transcription factors that regulate genes responsible for cell death and proliferation, and is frequently deregulated in cancer. Upon DNA damage, NF-κB targets include antiapoptotic genes, thus blocking cell death. Conversely, loss of NF-κB signaling renders cells more radiosensitive. In model systems of breast cancer, PARP1 inhibition resulted in diminished radiation-induced NF-κB binding to target gene loci. This decrease in NF-κB chromatin occupancy was not due to altered IκB degradation or NF-κB nuclear localization, but rather decreased radiation-induced NF-κB transcriptional activity (determined by reporter assay; ref. 84). Although the mechanism of this apparent coactivator function for PARP1 for NF-κB is not yet defined, the biologic consequence of PARP1 inhibition in breast cancer cell lines is an increase in radiation-induced apoptosis and radiosensitization.

In addition to modifying the response to externally applied radiation, PARP1 transcriptionally regulates systemic radiotherapy in selected contexts. Radioiodine is the only effective therapy for disseminated thyroid cancer, as the thyroid absorbs most of the iodine present in the body, but upon dedifferentiation, tumors no longer respond to this therapeutic modality due to loss of expression of human sodium-iodide symporter (hNIS). hNIS is a transmembrane protein that facilitates the concentration of iodide in both normal and transformed thyroid follicular cells. A number of potential mechanisms for loss of hNIS have been reported, including CpG island methylation and activation of a *trans*-acting repressor. Upon characterization of the *trans*-acting repressor, it was found that PARP1 is a constituent of the complex. PARP1 occupies the hNIS promoter, and PARP inhibition results in increased hNIS reporter activity as well as endogenous hNIS mRNA expression, suggesting that PARP1 enzymatic activity may repress hNIS expression (85). Although the mechanism and biologic consequence is unclear, PARP inhibition may sensitize disseminated refractory thyroid tumors to radioiodine.

Finally, with regard to the response to radiotherapy, PARP1 plays a key role in BER, and HR-deficient cells rely on BER as regulated by PARP1. In fact, among BRCA-deficient tumors, use of PARP inhibitors has demonstrated some efficacy due to synthetic lethality. It has been observed that under hypoxic conditions, there is downregulation of *BRCA1* and *RAD51* gene transcription via accumulation of a suppressive complex containing E2F4/p130 at regulatory loci. As such, it could be predicted that hypoxia would render cells sensitive to PARP inhibition. Consequently, it was found that colon and lung cancer cells under hypoxic conditions were more sensitive to PARP inhibition than cells under normoxic conditions. Inhibition of PARP activity resulted in diminished BRCA1 and RAD51 protein expression in cell models of lung cancer, breast cancer, and osteosarcoma. This suppression of BRCA1 and RAD51 could be reversed by either HPV E7 expression or p130 knockdown, and was associated with diminished E2F4 and p130 occupancy at the regulatory loci, indicating that PARP inhibitor-mediated regulation of BRCA1 and RAD51 is due, in part, to E2F4/p130-mediated suppression. Further mechanistic studies indicated that PARP inhibition results in an increase in E2F4/p130 complex formation and p130 hypophosphorylation, which inactivates its function. Further biologic studies demonstrated that PARP inhibition sensitizes cancer cells to radiation by suppressing DNA damage repair in a p130-dependent mechanism (86). Together, PARP1 not only regulates the DDR to radiation, but also the transcriptional events associated with this therapeutic modality, and utilization of pharmacologic PARP1 inhibitors may be clinically relevant in the administration of radiation.

Transcriptional Regulation in Hormone-Dependent Cancers

NRs are transcription factors that function in many processes, including homeostasis, development, reproduction, metabolism, and cancer. Hormone receptors act as

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ligand-dependent transcription factors, serving as the means by which steroid signals generate biologic responses. Given the fact that many cancers display aberrant NR signaling and have properties that make them amenable to pharmacologic targeting via endocrine therapy, NRs play a significant role in many cancer types. Several studies have examined the role of PARP1 in mediating tumor-associated NR activity.

In breast cancer, PARP1 elicits disparate functions in estrogen receptor (ER) biology, depending on ERα or ERβ status. ERα is a ligand-dependent nuclear hormone receptor, and when activated in the context of breast cancer serves a proproliferative role. ERα is expressed in approximately 70% of breast cancer cases, and serves as a therapeutic target for some metastatic patients with breast cancer (such as through the use of tamoxifen therapy, an ERα antagonist). However, not all patients respond uniformly, and all will eventually relapse, resulting in the generation of hormone-independent breast cancer that is resistant to endocrine therapy. Thus, understanding the mechanisms that regulate ER-mediated signaling in breast cancer is of importance. The transcriptional response to 17β-estradiol (E2) in MCF-7 breast cancer cells results in transient DSBs, and the subsequent recruitment of PARP1 and topoisomerase IIβ (TopoIIβ) to the promoters of ERα target genes. Abolishing either PARP1 or TopoIIβ function resulted in diminished ability of E2 to activate the expression of classic ERα target genes, demonstrating that in this context, PARP1 is required for ERα transcriptional activity (87). In contrast, ERβ exhibits antiproliferative and prodifferentiative functions in several organ systems, including lung, colon, prostate, and mammary gland. It has been suggested that the ratio of ERα to ERβ determines whether breast cancer tissue is proliferative and how the tissue will respond to hormone therapy (tamoxifen); however, the role of ERβ in breast cancer remains incompletely defined (88). A study that sought to illuminate the mechanism by which ERβ drives transcription in breast cancer cells determined that tamoxifen treatment of MCF-7 breast cancer cells served to protect cells from E2-induced oxidative DNA damage. This tamoxifen-induced protection was due to recruitment of ERβ to electrophile response elements (EpRE), which in turn induced the expression of antioxidative enzymes, including NQO1 (NAD(P)H quinone oxidoreductase), which required a number of cofactors, including PARP1. In fact, it was found that upon depletion of PARP1, the tamoxifen-dependent expression of antioxidative enzymes was compromised, demonstrating that in this context, PARP1 is potentially a coactivator for ERβ (89). Together, it seems that PARP1 is a positive regulator of both ERα and ERβ in models of breast cancer, and that this positive regulation requires TopoIIβ. As both ERα and ERβ play significant roles in breast cancer biology, future analyses of the biologic impact of PARP1 regulation of both ERα and ERβ in breast cancer is critical, due to the differential functions of these NRs.

Progesterone receptor (PR) function is activated by the ovarian steroid progesterone, and serves to regulate differentiation of the endometrium, maintenance of pregnancy, and proliferation of the mammary gland. Nuclear PR acts as a transcription factor, whereas cytosolic PR acts as a rapid signal transducer. Progesterone can stimulate proliferation independently of estrogen, and is considered a risk factor for breast cancer. In models of breast cancer, it has been demonstrated that PAR accumulates after progestin stimulation, indicating that the activation of PR induces PARP1 activity. It was found that progestin induced a physical interaction between cyclin-dependent kinase 2 (CDK2) and PARP1, followed by phosphorylation and increased enzymatic function of PARP1. Genome-wide analyses indicated that both CDK2 and PARP1 are enriched at PR-binding sites in response to progestin stimulation, and that the majority of PR-regulated genes required CDK2 and/or PARP1 for proper activation or repression (90). These data indicate that in models of breast cancer, progestin stimulates CDK2 to interact with and activate PARP1, and this complex serves as a coregulator of PR.

In prostate cancer, AR plays a key role in cell proliferation and maintenance of prostate cancer–associated phenotypes. AR serves as the target of first-line therapy for disseminated disease, but upon relapse, AR activity is resurgent despite continued therapeutic targeting. There are limited options for patients with castrate-resistant prostate cancer (CRPC). Therefore, defining novel means of AR regulation is of critical importance.

PARP1 is recruited to sites of AR transcriptional function, and PARP1 enzymatic activity is required for AR-driven gene expression and subsequent prostate cancer cell proliferation in both the context of hormone therapy–sensitive and CRPC models of disease. The decrease in AR activity in response to PARP inhibition was associated with diminished AR and PARP1 residency at sites of AR function, as well as altered capacity of androgen stimulation to elicit protranscriptional changes in histone modifications and chromatin architecture. Further analyses indicated that PARP1 enzymatic activity was increased as a function of transition to CRPC, implying a role for PARP1 in the evolutionary progression of prostate cancer. Ultimately, pharmacologic inhibition of PARP1 resulted in diminished AR activity and diminution of subsequent tumor growth *in vivo* and *ex vivo*, implicating PARP1 enzymatic activity in the maintenance of the CRPC phenotype *in vivo.* Together, these data demonstrate that in the context of prostate cancer, PARP1 seems to serve as an activator of AR function and effector of downstream biologic consequences. As described above, PARP1 also regulates the activity of ETS transcription factors in models of prostate cancer, which is of clinical significance, given the high percentage of prostate tumors that harbor fusions that put ETS expression under the control of AR activity (as through the *TMPRSS2–ERG* fusion). As such, the studies demonstrating regulation of both AR and ETS transcription factors by PARP1 are now being translated into the clinic in a trial combining PARP inhibition and an AR-directed therapeutic (abiraterone acetate) for patients with metastatic CRPC (NCT01576172; ref. 91). Combined, the studies outlined above implicate PARP1 in the regulation of several NRs that have significant roles in human cancer.

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With respect to NRs in cancer, PARP1 has an apparent positive role in regulating transcriptional events. As such, further analyses of mechanisms of PARP1 responsive transcription by NRs and the biologic impact of PARP inhibition should be considered.

Conclusions and Future Directions

Modifying transcription factor function has long been a goal of cancer research, with some successes, especially in the context of ligand-dependent transcription factors such as NRs. Although it has been long understood that selected transcription factor activities can drive tumor formation and progression, many have proved to be difficult to develop modalities to target them directly, but understanding mechanisms of how "druggable" enzymes regulate transcription factors may yield clinically translatable results. Gains in our understanding of how PARP1 regulates transcription in human cancer may bring new appreciation of the mechanisms that support aberrant transcriptional events and downstream processes in human disease. The transcriptional roles of PARP1 should be considered not only in future design of basic and clinical investigation in the utility of PARP inhibitors for cancer therapy, but also the impact that PARP1 has on transcription in the way that previous studies are interpreted. Although there is obviously no unifying theory of transcriptional regulation in cancer by PARP1, given the divergent and context-specific functions and outcomes of PARP1-dependent transcription (Fig. 2), findings discussed herein underscore the major impact of PARP1-mediated transcriptional control on human tumor biology. Attaining greater mechanistic insight to these events, discerning the cause and effect of the context-specific PARP1 functions, and using this knowledge for the development of new trials is likely to have significant clinical impact.

Although there have been major advances in understanding of PARP1 transcriptional regulatory functions, key questions remain that must be addressed to delineate the complex role of PARP1 in human malignancy. *First*, what are the context-dependent molecular determinants of transcriptional regulation by PARP1? Although it is apparent that PARP1 regulates transcription, the molecular mechanisms by which PARP1 selectively supports or represses transcription are poorly understood and the results to date are disparate in nature. It is not known whether the transcriptional roles for PARP1 outlined above may be universal, and it is not yet established which divergent roles of PARP1 influence the tumorigenic program. *Second*, do other PARP family members compensate for PARP1 in transcriptional regulation, and how are other PARP family members affected by PARP inhibition? PARP1 and PARP2 are the closest family members, and double-null mice are embryonic lethal, speaking to the overlap and importance of PARP1 and PARP2. However, as of yet it has not been determined whether other PARP family members can contribute to transcription to a similar degree and in similar contexts as PARP1. In addition, some of the PARP inhibitors in trial for human malignancies exhibit less specificity than may be desired. As such, determining the impact of these drugs on other PARP members *in vivo*, and assessment of downstream cellular and biologic consequence would be of great value. *Third*, is it possible to specifically target the transcriptional regulatory function of PARP1 in cancer therapy? Recent evidence suggests that specific modules of PARP1 regulate allosteric communication, and abrogation of this communication supports the contention of context-dependent transcriptional regulation by PARP1, but not the DDR function of PARP1 (92). Given these compelling findings, pursuit toward more specifically targeting PARP1 transcriptional regulation is currently underway. *Fourth*, given the significant body of evidence suggesting that PARP1 controls critical transcriptional events in models of cancer, do these events alter cancer biology, in the laboratory and in the clinic? Many of the studies described herein put forth compelling mechanistic observations about how PARP1 regulates specific transcriptional events in cancer, yet few assess causation to more fully understand the true impact of PARP1, and by extension, PARP inhibitors in the field of cancer biology, and these deficiencies must be addressed. *Fifth*, within the field, there is a controversy as to whether transcription-associated DNA damage is a true phenomenon. Some literature points to transcription causing transient DSBs (87, 93–97). However, whether this is a true biologic outcome, what the explicit role that PARP1 plays in causing/maintaining these breaks remains unclear. *Finally*, does the concept of transcriptional regulation by PARP1 alter the interpretation and implications of ongoing and concluded oncology clinical trials, and can the transcriptional regulatory roles of PARP1 be fully harnessed for clinical benefit? Initial PARP inhibitor clinical trials were rationally developed on the basis of the synthetic lethal interaction of HR deficiency and PARP inhibition. However, given the complex, diverse, and context-specific roles of PARP1 in regulating key transcriptional events in cancer, the implications thereof for therapeutic response should be considered. In conclusion, PARP1 functions to regulate many key transcriptional events in cancer biology, and while much is known, further mechanistic insight may lead to better utilization of PARP inhibitors in human malignancies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed. The Editor-in-Chief of *Molecular Cancer Research* is an author of this article. In keeping with the AACR's Editorial Policy, the paper was peer reviewed and an AACR Journals' Editor not affiliated with *Molecular Cancer Research* rendered the decision concerning acceptability.

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