p27 as a Transcriptional Regulator: New Roles in Development and Cancer

Seyedeh Fatemeh Razavipour[1], Kuzhuvelil B. Harikumar[2], and Joyce M. Slingerland[1]

ABSTRACT

p27 binds and inhibits cyclin-CDK to arrest the cell cycle. p27 also regulates other processes including cell migration and development independent of its cyclin-dependent kinase (CDK) inhibitory action. p27 is an atypical tumor suppressor–deletion or mutational inactivation of the gene encoding p27, *CDKN1B*, is rare in human cancers. p27 is rarely fully lost in cancers because it can play both tumor suppressive and oncogenic roles. Until recently, the paradigm was that oncogenic deregulation results from either loss of growth restraint due to excess p27 proteolysis or from an oncogenic gain of function through PI3K-mediated C-terminal p27 phosphorylation, which disrupts the cytoskeleton to increase cell motility and metastasis. In cancers, C-terminal phosphorylation alters p27 protein–protein interactions and shifts p27 from CDK inhibitor to oncogene. Recent data indicate p27 regulates transcription and acts as a transcriptional coregulator of cJun. C-terminal p27 phosphorylation increases p27-cJun recruitment to and action on target genes to drive oncogenic pathways and repress differentiation programs. This review focuses on noncanonical, CDK-independent functions of p27 in migration, invasion, development, and gene expression, with emphasis on how transcriptional regulation by p27 illuminates its actions in cancer. A better understanding of how p27-associated transcriptional complexes are regulated might identify new therapeutic targets at the interface between differentiation and growth control.

p27 Is a Ubiquitously Expressed Cell-Cycle Inhibitor

The *CDKN1B* gene encodes p27, a cyclin-dependent kinase inhibitor (CDKi) of the kinase inhibitory protein (Kip) family. CDKis mediate cell-cycle inhibition. p27 is ubiquitously expressed and integrates mitogenic and growth inhibitory signals to govern normal cell-cycle progression (1). p27 can inhibit the catalytic activity of cyclin D-, E-, A-, and B-CDK complexes (2) by interacting with both cyclin and CDK subunits via its N-terminal domain, which is conserved among Kip family members (p21, p27, and p57; refs. 1, 3).

Antiproliferative and differentiation signals increase p27 to mediate cell-cycle arrest (2, 4–7). In normal cells, p27 levels are tightly regulated across the cell cycle. An increase in p27 by 2- to 3-fold is sufficient to fully inhibit G_1–S-phase cyclin-CDKs (8). In G_0-phase and early G_1-phase, p27 protein translation and stability are maximal (9–13) and it inhibits cyclin E-CDK2 (2, 14). A progressive decline in p27 is required to relieve the inhibition of cyclin E- and cyclin A–bound CDK2 and enable transcription of genes required for G_1- to S-phase progression (1). Transient C-terminal p27 phosphorylation by PI3K/AKT in mid-G_1-phase facilitates assembly and nuclear import of D-type cyclin-CDKs (15), permitting their activation (3, 15, 16). p27 proteolysis increases during G_1-phase progression via a number of mechanisms. Phosphorylation at p27T187 by cyclin E or cyclin A–bound CDK2 triggers p27 turnover by promoting its polyubiquitination and degradation by the SCF^{Skp2} ubiquitin ligase complex [S-phase kinase associated protein 1 (SKP1)/Cullin/F-Box protein: SKP2; refs. 17–19]. But how does the target kinase, cyclin E–bound CDK2, phosphorylate its own inhibitor? In G_1-phase, Src family kinases phosphorylate p27 in its CDK inhibitory domain at Y74, Y88, and Y89 (20, 21). Phosphorylation of p27Y88 within the 310-helix that binds the ATP pocket in CDK2, leads to ejection of p27 from the catalytic cleft of CDK2, permitting kinase activation (20, 21). CDK2 can then phosphorylate p27 at T187 to trigger p27 proteolysis at the G_1–S-phase transition. p27 is also degraded independently of T187 phosphorylation (see **Fig. 1A**). In early G_1-phase, p27 phosphorylation at S10 (22, 23) leads to its export to the cytoplasm. Cytoplasmic p27 can be ubiquitylated by the ubiquitin ligase Kip1 ubiquitylation-promoting complex to mediate its degradation (24).

p27 Acquires Novel Functions through C-terminal Phosphorylation by PI3K Effector Kinases

C-terminal p27 phosphorylation regulates p27 function. PI3K promotes growth, survival, and motility (25–27) of both normal and cancer cells (28). PI3K activates downstream kinases, including AKT, SGK, 70 kDa S6 kinase ($p70^{S6K}$), and 90 kDa ribosomal S6 kinase ($p90^{RSK}$; refs. 26, 27, 29). These in turn phosphorylate p27 at T157 (30–32) to delay p27 nuclear import (30, 33) and at T198 (34, 35) to stabilize p27pT157pT198 (hereafter p27pTpT; refs. 36, 37).

p27 plays cell-cycle–independent actions to regulate cell motility (38, 39). Early work showed transduction of p27–TAT fusion protein into HepG2 cells increased cytoplasmic p27 and promoted cell migration (40). When p27null mouse embryo fibroblasts (MEF) were found to have impaired cell motility, this led to the discovery that p27 binds and inhibits RhoA-ROCK, leading to

[1]Breast Cancer Program, Lombardi Comprehensive Cancer Center, Department of Oncology, Georgetown University, Washington DC. [2]Cancer Research Program, Rajiv Gandhi Centre for Biotechnology (RGCB), Thiruvananthapuram, Kerala, India.

S.F. Razavipour and K.B. Harikumar contributed equally as co-first authors for this article.

Corresponding Author: Joyce M. Slingerland, Lombardi Comprehensive Cancer Center, Department of Oncology, Georgetown University, 3970 Reservoir Rd, Rm E212, Washington DC 20057. Phone: 202-687-4388; Fax: 202-687-3341; E-mail: js4915@georgetown.edu

Cancer Res 2020;80:3451–8

doi: 10.1158/0008-5472.CAN-19-3663

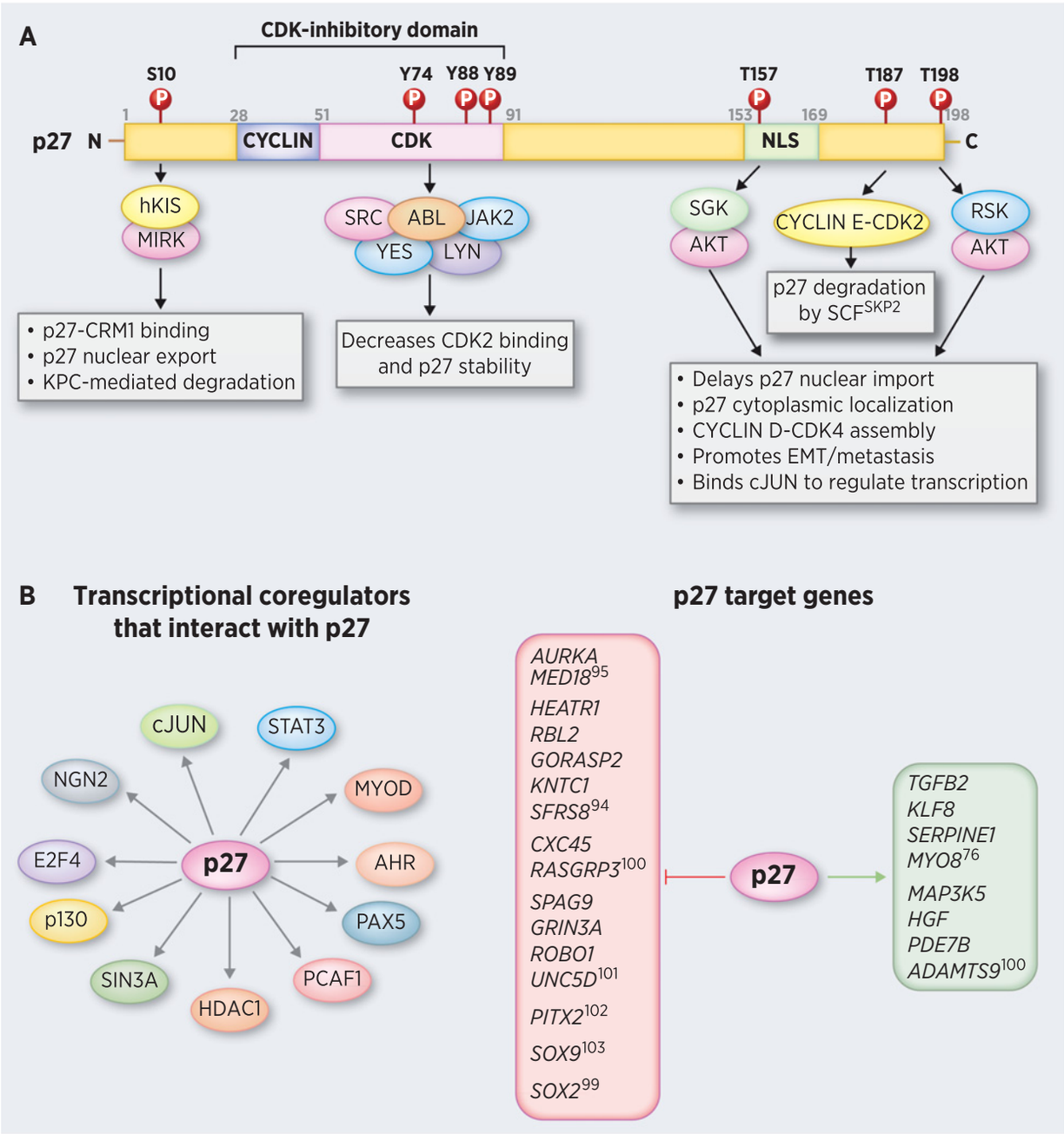

Figure 1.

A, Schematic of p27 domains and phosphorylation sites. Kinases that phosphorylate p27 are indicated. **B,** Transcription factors that interact with p27 and p27 target genes validated by ChIP-PCR are shown (the superscripts on p27 target genes indicate the relevant references).

destabilization of the actin cytoskeleton and increased cell motility (38, 39). This p27 action is independent of cyclin/CDK binding, because the motility of p27null MEFs is restored equally by either WTp27 or by a p27CK$^-$ mutant that cannot bind cyclins and CDKs (38, 39). p27 phosphorylation at T198 promotes its interaction with RhoA and RhoA-ROCK1 inhibition (41, 42) to drive cell motility. While the precise role of p27-mediated cytoskeletal changes in normal cells are not fully known, these might serve to remodel cell shape in G_1-phase to permit later changes during mitosis and cytokinesis.

p27 is also expressed in cortical neurons and neuronal progenitors and regulates interneuron migration. p27 plays roles in neuronal development and axonal transport via several mechanisms. Defects in the kinetics of nucleokinesis and tangential neuronal migration were observed in p27null mice (43), potentially through loss of p27/RhoA binding. p27 also promotes neuronal microtubule polymerization

during neurite outgrowth (44, 45). p27 also appears to bind and stabilize alpha tubulin acetyl transferase 1 to promote microtubule acetylation and stability to regulate axonal transport (46). In addition to its effects on cell motility, p27 plays a number of roles that are independent of CDK inhibition to regulate autophagy, apoptosis, stem/progenitor fate, and cytokinesis (37, 47, 48).

Loss of CDK Inhibition by p27 in Cancers

p27 loss through decreased synthesis and excess proteolysis

Oncogenic activation of the SRC, MAPK, and PI3K signaling pathways deregulate p27 in cancers (1, 49). While high nuclear p27 levels restrain normal cell proliferation (50), p27 is nearly always deregulated in cancers (20). Human cancers often show reduced nuclear p27 (50–53) and this is associated with poor patient prognosis (1). This occurs predominantly through activation of SCF^{SKP2}-dependent p27 proteolysis (17, 24, 36). Activation of ABL, LYN, LCK, and FYN in lymphoma and other hematopoietic malignancies also promotes p27 proteolysis (54). In addition, SRC activation (20) and *ERBB2* amplification (55) are associated with reduced p27 in human breast cancer. p27 loss in human cancers can also result from oncogenic overexpression of miRNAs that impair p27 translation (reviewed in ref. 12). p27 is a key target of the miR-221/222 in multiple malignancies including glioblastoma (56, 57), triple-negative breast (58), hepatocellular (59), and papillary thyroid carcinoma (60). Other miRNAs also target p27 including miR-196a in cervical cancer (61), miR-24 in prostate cancer (62), miR-152-3p in chronic myelogenous leukemia (63), miR-148a in myeloma (64), and miR-199-a in osteosarcoma (65). p27 translation can also be regulated by long noncoding RNAs via ribonucleoprotein complexes (66), but the relevance of this mechanism to p27 loss in cancers is not known.

Constitutive C-terminal phosphorylation via oncogenic PI3K pathway activation

The PI3K pathway is oncogenically activated in a majority of human cancers (28). PI3K/AKT-activated cancers have constitutively C-terminally phosphorylated p27 that accumulates aberrantly in both the cytoplasm and nucleus and binds novel proteins to drive tumor progression (30–32). We and others showed AKT can phosphorylate p27 (30–32), impair nuclear import, and a highly stable p27 accumulates in the cytoplasm in PI3K-activated human breast cancers and is associated with poor patient outcome (30, 67). Cytoplasmic p27 was also observed in cancer models with oncogenic activation of Ras, PKC, or Pim kinases (68–71). Notably, PI3K inhibition restored nuclear localization of p27 in a K-Ras–activated lung cancer model (72). Cytoplasmic p27 is associated with increased metastasis and poor survival in a number of cancer types (1, 67). Indeed, overexpression of $p27CK^-$ coupled to a triple-nuclear export signal increased melanoma metastasis *in vivo*, suggesting p27 might have oncogenic actions independent of CDK inhibition (73). Activation of AKT/PI3K and accumulation of p27pTpT has been shown to increase tumor metastasis in several cancer models including breast and urothelial cancers (74–76). While the oncogenic action of C-terminally phosphorylated p27 was initially thought to result from cytoplasmic mislocalization (30), increased cyclin D-CDK4 assembly (3, 15) or RhoA/ROCK1 inhibition and cytoskeletal changes causing greater tumor invasion (38, 39), recent work has identified additional p27pTpT-interacting partners and novel oncogenic actions (see **Figs. 1B** and **2**).

Increasing data indicate that p27pTpT acts as a transcriptional regulator to drive metastasis (75, 76). When p27pTpT accumulates due to PI3K activation, it associates with transcription factors STAT3 (75) and cJun (76) to drive transcription programs of epithelial–mesenchymal transition (EMT) and metastasis (reviewed below). cJun is a known regulator of tissue morphogenesis and multi-organ tissue differentiation (77). The finding that p27-cJun–driven genes govern developmental processes may illuminate work from 20 years ago that implicated p27 as a regulator of tissue differentiation in normal development. Before actions of p27 as a transcriptional regulator are discussed, we will briefly review lessons from mouse models on p27 action in differentiation and development.

p27 Plays Cyclin-CDK–Independent Roles in Development

$p27^{-/-}$ mice have a phenotype of gigantism due to multi-organ hyperplasia, and maldevelopment of retina, central nervous system, pituitary, and ovary (78–80). This has been attributed to failure to arrest the cell cycle during differentiation (78–80). Mice lacking p27 uniformly exhibit tumor formation in the pituitary (78–80); and haploinsufficient animals are carcinogen sensitive (81). Spleen and thymus enlargement in p27-null mice are associated with an expansion of committed hematopoietic progenitors of granulocytic and erythroid lineages, without increase in pluripotent hematopoietic stem cells (80, 82), and spontaneous T-cell lymphoma emerge in 6%. $p27^{-/-}$ mice also show maldevelopment of neural structures, retina, and pituitary, and pituitary adenoma cause premature death of nearly all mice at approximately 10 months (78–80). Notably, knockin of a cell-cycle defective p27 mutant that cannot bind cyclin or CDKs ($p27CK^-$) failed to correct the large animal size, providing additional support for the notion that the CDK inhibitory function of p27 somehow coordinates stem/progenitor expansion in various tissues with cell-cycle arrest at differentiation (83). Furthermore, some developmental defects of the p27-null phenotype were corrected in the $p27CK^-$ knockin, indicating that some developmental actions of p27 are CDK independent (83). The $p27CK^-$ knockin mice showed stem cell expansion, hyperplasia, and neoplasia in the lung, not seen in $p27^{-/-}$ and a greater frequency of hyperplasia of pituitary, adrenal, spleen, and thymus and more T-cell lymphomas (20%; ref. 83). The widespread differentiation defects and increased progenitor self-renewal in multiple tissues, suggested $p27CK^-$ expression without cell-cycle restraint might misregulate progenitor/stem cells. The developmental phenotypes of p27-null and $p27CK^-$ knockin mice were initially interpreted in light of p27's known CDK inhibitor action. However, CDK2 loss did not compensate the p27null phenotype in the double knockout (84, 85) and subsequent studies soon showed further evidence for CDK-independent p27 actions in differentiation.

The p27 homologue in *Xenopus*, Xic1, is required for normal muscle (86, 87) and neuronal differentiation (88–90). This prodifferentiation function is cell-cycle independent (86, 87), because Xic1 is required prior to growth arrest during differentiation and because a mutant xic1, which cannot bind cyclin-CDKs, can compensate for Xic1 loss to promote differentiation. p27 interacts with several transcription factors either genetically or physically to govern differentiation. p27 interacts in a cyclin-CDK–independent manner with Nrf2, and binds and stabilizes Neurogenin 2 to regulate neuronal development in both frogs and mice (88–90). p27 cooperates with myogenic transcription factor, MyoD (86), to drive myogenesis (86, 87). p27 is also involved in the maintenance of muscle stem cells (MuSC). p27, together with the upstream activator AMPK, prevents apoptosis of aged MuSCs by enhancing autophagy (91). p27 also interacts functionally and genetically with transcription regulator, p130, to promote

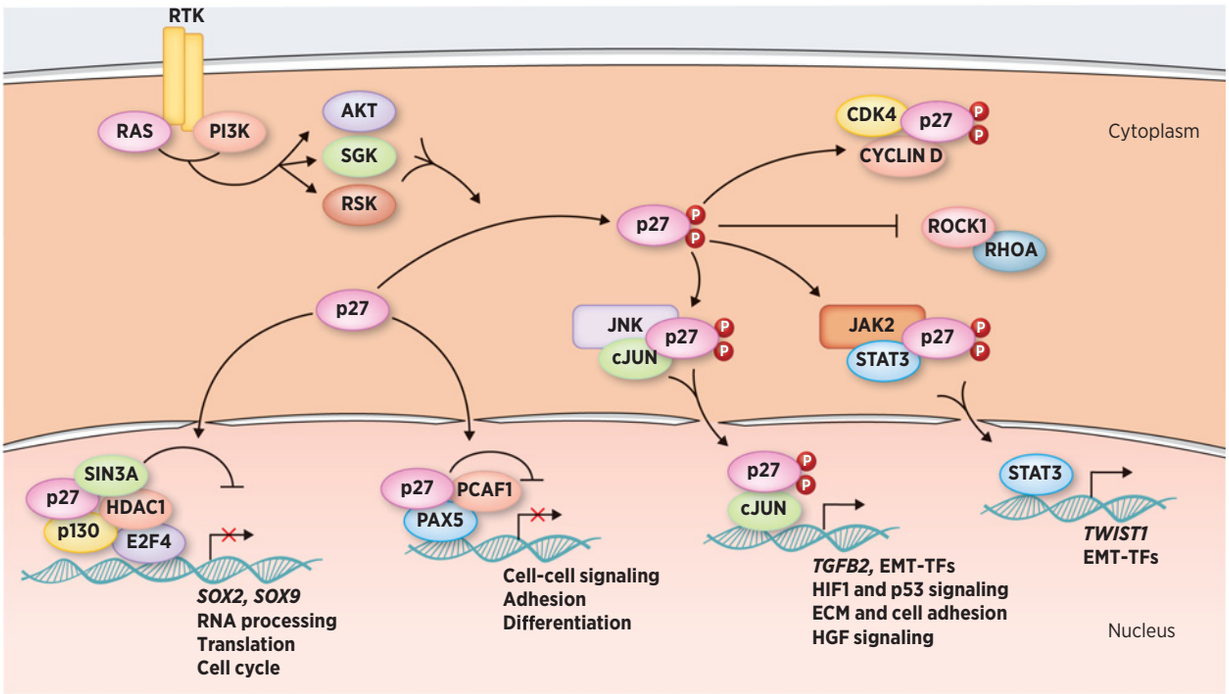

Figure 2.
Cartoon depicts signaling pathways linked to transcriptional regulation by p27 and interacting coregulators. ECM, extracellular matrix; HGF, hepatocyte growth factor; TF, transcription factor.

endochondral ossification (92). In keratinocyte precursors, antisense oligonucleotides to p27 disrupted the expression of differentiation markers but did not prevent growth arrest, indicating that normal keratinocyte differentiation is p27 dependent but does not require its CDK inhibitory action (93). Taken together, these findings indicate that p27 controls cell proliferation and organ size (78–80) through both cell-cycle–dependent and -independent mechanisms to regulate normal tissue progenitor expansion and differentiation. Because the p27null phenotype was not compensated by CDK2 knockout (84, 85) and some developmental defects in neurogenesis and myogenesis of p27null tissues can be compensated by expression of p27CK$^-$ (86, 88, 90), it appears that p27 has developmental actions independent of its cyclin-CDK binding. In the section below, we review emerging data that indicate a novel role for p27 in regulation of transcription. The developmental defects and organ overgrowth of p27null mice might reflect a normal role for p27 to govern gene programs that restrain tissue stem or progenitor cell self-renewal during differentiation and to integrate this with cell-cycle arrest.

Evidence for a Role for p27 as a Transcriptional Corepressor

Increasing data suggest that some of the developmental effects of p27 might result from novel actions on transcription (see **Figs. 1B** and **2**). A limited genomic survey using chromatin immunoprecipitation (ChIP)-on-chip in quiescent MEFs showed p27 binds gene promoters as a putative corepressor with p130, E2F4, HDAC1, and SIN3A (94). Comparison of p27-annotated sites with genes differentially expressed in G_0-phase wild-type (WT) MEFs versus p27null MEFs indicated that p27-target genes regulate splicing, mitochondrial function, translation, and cell cycle. Notably, interaction of p27 with p130 required the C-terminal portion of p27 and further assays support a model in which p130 recruits p27 to DNA and p27 is required to nucleate E2F4 and other corepressors (94).

It is not clear whether some transcriptional effects of p27 involve interaction with cyclin-CDKs. p27 recruitment to two putative repressed gene targets *AURKA* and *MED18* identified by ChIP-on-chip (94), was evaluated across the cell cycle (95). At both sites, p27–promoter interaction decreased as p27 levels declined during progression from G_0-phase to mid-G_1-phase in synchronized NIH3T3 cells (95). ChIP-PCR also demonstrated individual recruitment of each of cyclins D2, D3, and CDK4 to the sites of p27 association, peaking in mid-G_1-phase. Interactions of cyclin D2, D3, and CDK4 with these promoters decreased with p27 knockdown and were not restored by transfection of p27CK$^-$, suggesting a need for D-type cyclin and CDK4 binding to p27 for their recruitment to chromatin. While these data raise the intriguing possibility that some transcriptional roles of p27 might involve cyclin-CDKs, further work is required to establish whether p27-dependent D-Cyclin-CDK4 recruitment reflects a tripartite complex at these promoters and whether these complexes indeed regulate target gene expression (95).

Further evidence for a role for p27 in gene repression came from MEFs and embryonic stem (ES) cells. p27$^{-/-}$ MEFs cells were shown to have higher basal expression of *SOX2*, suggesting p27 might act as *SOX2* repressor. Sox2 is a critical ES cell transcription factor that maintains ES pluripotency and self-renewal (96) and drives stem cells in many different cancers (97, 98). *SOX2* has an intronic regulatory element (SRR2). p27 interaction with this *SOX2*-SRR2 and each of p130, E2F4, and SIN3A led to *SOX2* repression (99).

A genomic survey in G_0-phase–arrested MEFs showed p27 is recruited to chromatin at consensus motifs of several other developmentally important transcription factors including PAX5 and MyoD,

and p27 was shown to coprecipitate with each of these factors (100). p27 showed greater recruitment to distal putative intronic-binding sites compared with promoter proximal sites in G_0-phase MEFs. Gene ontology analysis of protein coding p27 targets showed enrichment of pathways governing cell adhesion, differentiation, transcriptional regulation, and morphogenesis among others (100). Further work in growth arrested HCT116 colon cancer cells, showed up to half of p27-associated protein-coding gene targets were also sites of recruitment of p300/CBP-associated factor (PCAF). Correlation of ChIP-sequencing with gene expression following knockdown of each of *CDKN1B* and *PCAF* suggested that p27 acts predominantly as a PCAF corepressor to decrease target gene expression (101).

In p27null mice, p21 levels are increased and p21 appears to compensate for some cell-cycle defects of p27 loss (78–80). A recent report suggests that p27 downregulates expression of the p21 gene, *CDKN1A*, by repressing *PITX2*, a transcriptional activator of *CDKN1A*. Both *CDKN1A* and *PITX2* expression were increased in $p27^{-/-}$ MEFs compared with WT MEFs. ChIP-PCR showed p27 recruitment to the *PITX2* enhancer in association with loss of *PITX2* expression and loss of p21. E2F4 was also recruited to this *PITX2* site and E2F4 depletion also increased *PITX2* expression supporting a model of p27-E2F4–mediated *PIXT2* repression (102).

In a mouse model of K-Ras–driven pancreatic cancer, loss of p27 accelerated tumor development and decreased survival. Pancreatic acinar cells in $p27^{-/-}$ mice showed a disruption of apical basal polarity, premalignant acinar to ductal metaplasia, and reexpression of ductal progenitor markers, including Sox9. In the K-Ras–activated pancreatic cancer line, PANC1, WTp27 was shown to be recruited to the *SOX9* promoter and to decrease its promoter activity in reported assays (103). The recruitment of corepressors was not characterized. The oncogenic cooperation between K-Ras and p27 loss was attributed in part to de-repression of *SOX9* (103).

These studies, taken together, provide a body of work supporting a role for p27 in transcriptional repression of pathways governing cell adhesion, differentiation, and development. However, most of the studies above have provided little or no data to demonstrate the functional significance of p27-driven gene repression *in vivo*. The changes in pancreatic acinar polarity in $p27^{-/-}$ mice and more rapid emergence of $p27^{-/-}$ X-mutant *K-RAS*–driven pancreatic cancer were associated with, but not shown to be caused by, loss of *SOX9* repression by p27 (103). In the case of *SOX2* repression, p27null MEFs more readily undergo induced pluripotency due to higher endogenous levels of *SOX2* expression (99). However, the functional consequences of p27-mediated transcriptional repression of *SOX9, SOX2*, or other gene targets *in vivo* in development and cancer have yet to be demonstrated.

Oncogenic Partnerships between p27pTpT and Other Transcription Factors

p27 activates STAT3 to mediate TWIST1 induction, EMT, and metastasis

In MCF12A human mammary epithelial cells, a CDK-binding defective p27pT157pT198-phosphomimetic, p27CK-DD, induced EMT, but the $p27CK^{-}$ allele lacking these phosphomimetic mutations did not (75). A comparison of sister breast cancer lines with low and high metastatic ability, respectively, showed the highly metastatic lines had activated PI3K and high p27pTpT (67). p27 depletion mimicked mTOR inhibition and abrogated the excess bone metastasis in the MDA-MB-231-1833 model compared with parental MDA-MB-231 (67). C-terminally phosphorylated p27 was shown to activate gene programs of EMT: in highly metastatic breast and bladder cancer lines, p27 depletion reduced expression of EMT transcription factors including T*WIST1* and *TGFB2*, decreased activated STAT3 (pSTAT3), and reduced invasion and lung metastasis (75). In contrast, p27CK-DD transduction into low metastatic breast and bladder cancer cell lines activated pSTAT3, to upregulate TWIST1 and increase lung metastasis *in vivo*. Proteome analysis of 747 primary breast cancers showed p27pT157 levels correlated strongly with p27pT198, and with both pSTAT3 and PI3K activation (75). Thus, PI3K activation leads to increased p27pTpT to drive p27/STAT3 association, STAT3 activation, and STAT3-induced *TWIST1*, EMT, and metastasis (75). Further analysis of p27–STAT3 partnerships in transcriptional regulation is in progress.

p27 binds and transcriptionally coregulates cJun to drive programs of tumor progression

A recent study revealed a novel oncogenic cooperation between PI3K and cJun pathways: p27 phosphorylation by PI3K-activated kinases stimulates p27 association with cJun. p27 and cJun were shown to be corecruited to chromatin, leading to activation of transcription programs of cell motility and EMT to drive tumor metastasis (76). p27 thus emerges as a novel cJun coregulator, whose chromatin association is governed by C-terminal p27 phosphorylation. In cancer cells with high endogenous p27pTpT or expressing p27CK-DD, cJun was activated and interacts with p27. p27–cJun complexes colocalized to the nucleus as shown by coprecipitation in fractionated cell lysates and proximity ligations assays. Sequential ChIP-qPCR with anti-cJun and re-ChIP with anti-p27 antibodies showed both are corecruited to *TGFB2* to drive its expression. Comparison of global p27 and cJun chromatin association with gene expression showed p27 and cJun are corecruited broadly to chromatin and, in highly metastatic cancer models, upregulate target genes critical for cell adhesion, cytoskeletal regulation, TGFβ pathway activation, and oncogenic signaling. The most frequent transcription factor consensus motif bound by p27 was AP1/cJun, but other developmentally important and growth regulatory transcription factor binding motifs were also observed. Evaluation of binding within 5 Kb of transcription start sites showed p27-cJun target genes were differentially regulated, either up or down in the highly metastatic versus low metastatic lines. C-terminal p27 phosphorylation increased its interaction with cJun and cJun-p27 corecruitment to chromatin.

Profiles of target genes repressed by p27-cJun suggest p27pTpT might repress differentiation pathways. Over half of cJun-binding sites were cooccupied by p27, and cJun recruitment appeared to be p27 dependent, because cJun recruitment to a majority of cooccupied chromatin sites decreased dramatically with p27 depletion. Metastasis of orthotopic mammary tumors was markedly reduced by either p27, *JUN*, or *TGFB2* depletion in the highly metastatic lines, showing the functional importance of p27-cJun–driven gene programs to metastasis (76). Finally, human breast cancers with high p27pT157 protein showed differential expression of p27-cJun target genes compared with cancers with low p27pT157. Both high p27pT157 and upregulation of p27-cJun targets were prognostic of poor patient outcome, underlining the biologic relevance of p27/cJun-driven gene programs to disease progression (76).

Our perspective of p27 deregulation in cancers has been expanded by the discovery of the novel roles of p27 in transcription. Disruption of p27 action can occur through excess proteolysis or miRNA-mediated loss of p27 translation, but also via other mechanisms. The oncogenic effects of p27 to disrupt the cytoskeleton and to upregulate the assembly and activation of D-type cyclins are not the only or

potentially even major consequences of constitutive C-terminal p27 phosphorylation in the context of PI3K activation. Up to 60% of human cancers show some level of PI3K pathway activation (104). In addition to p27-mediated effects on the actin cytoskeleton in cancers, C-terminal p27 phosphorylation in cancers would lead to profound changes in gene expression programs, disrupting adhesion and activating EMT and other oncogenic gene programs to drive metastasis. Further work is required to understand the complexity of the transcriptional machineries that interact with p27 in normal development and malignancy.

Conclusion

Our understanding of the transcriptional roles of p27 is still in its infancy. In quiescent normal MEFs, p27 appears to partner with p130, E2F4 to recruit HDAC1, and SIN3A to restrain gene expression (94, 99). In cancer models, p27 can activate both STAT3 and cJun to promote gene programs of EMT and metastasis (75, 76). Indeed, p27-cJun complexes appear to play roles to both activate and repress target genes, but mechanisms governing gene selection and induction versus repression are not yet known. p27 has been shown to interact with MyoD, NRF2, PAX5, and cJun and other developmentally important transcription factors (see **Figs. 1B** and **2**; refs. 76, 90, 100, 102). That p27 regulates gene targets governing cell migration and EMT also supports potential transcriptional roles during development. It will be of interest to determine how the transcriptional roles of p27 might govern differentiation in different tissues and contribute to the phenotypes of p27null and p27CK$^-$ mice.

The induction of broad pro-oncogenic gene programs by constitutive PI3K-driven p27–cJun interaction on chromatin targets offers a new explanation for the profound effects of p27pTpT on tumor metastasis. It will be of interest to determine whether changes in phosphorylation across the normal cell cycle govern changes in p27's role in transcriptional regulation and whether any of these trans-regulatory completes involve associated cyclin-CDKs.

In normal cells, AKT is transiently, periodically activated in early G_1-phase and this is associated with transient accumulation and loss of p27pTpT (15). This raises the possibility that cyclic C-terminal p27 phosphorylation might regulate periodic target gene selection and contribute to G_1-phase progression. Further work in normal cell types will be required to evaluate how and which periodic phosphorylation of p27 at S10 in early G_1-phase, T157 and T198 shortly thereafter in mid-G_1-phase, and other sites might govern interaction with different transcriptional machineries to affect target gene expression or repression. A better understanding of how p27–cJun transcriptional complexes are regulated in normal tissue development and how these go awry in cancer might identify new therapeutic targets to be exploited for tissue regeneration and illuminate other aspects of human diseases, including cancer that arise at the interface of differentiation and growth control.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

This work was funded by DOD BCRP grant number W81XWH-17-1-0456 to J.M. Slingerland.

Received November 26, 2019; revised March 25, 2020; accepted April 21, 2020; published first April 27, 2020.

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