CANCER BIOTHERAPY AND RADIOPHARMACEUTICALS
Volume 27, Number 7, 2012

DOI: 10.1089/cbr.2010.0802

**Review**

# A Crosstalk Imbalance Between $p27^{Kip1}$ and Its Interacting Molecules Enhances Breast Carcinogenesis

Weiwei He,[1] Xueting Wang,[2] Longbang Chen,[1] and Xiaoxiang Guan[1]

**Abstract**

$p27^{Kip1}$ (p27) is an inhibitor of cyclin/cyclin-dependent kinase complexes, the nuclear loss of which indicates poor prognoses in various solid tumors. In breast cancer cells, the p27 expression level usually decreases during tumor development and progression. In addition, p27 cytoplasmic mislocalization has been reported, but the exact molecular mechanisms remain unclear. Studies have indicated that its phosphorylation status is the key regulator and that several signal transduction pathways are involved in the regulation of both the expression and distribution of p27. To further understand the signals involved, the differences in the profiles of interacting proteins between tumor and normal cells should be elucidated. It is well known that p27 has various interacting partners, such as cyclin, cyclin-depend kinases, CRM1, Jab1, SKP2, and Spy1. Assays used to profile these proteins show differing intracellular p27 expression and localization depending on the cell-cycle phase. We hypothesize that the imbalance of crosstalk between p27 and the other molecules involved in the same signaling pathways plays an indispensable role in breast cancer carcinogenesis.

**Key words:** breast cancer, interacting protein profiling, p27, phosphorylation, signal transduction

## Introduction

Cell-cycle disruption is a hallmark of cancerous cells.[1] p27 is a member of the cyclin-dependent kinase (CDK) inhibitor family, acting as a potent tumor suppressor in a variety of human cancers, and is a key cell-cycle regulator.[2–4] To date, studies have shown that p27 is involved in cell differentiation, proliferation, apoptosis, cell-cell adhesion, and growth inhibition.[5,6] Breast cancer is one of the most common cancers in women. Solid tumors have heterogeneous characteristics and exhibit variability in patient survival rates that are dependent on subtype. Moreover, a prolonged period of time exists during which relapse is possible but uncertain. There are currently several approved therapies for treating breast cancer, and the availability of clinical samples from large patient cohorts has contributed to the prolific research undertaken in the field of breast cancer.[7] Many studies have shown that the level of p27 expression decreases during carcinogenesis, correlating with poor prognosis. p27 is an independent prognostic factor in breast cancer, and inhibiting its deregulation has proved to be an effective target in breast cancer therapy.[8,9] Moreover, there is considerable evidence that p27 inactivation is a fundamental step in the development of malignancies.[10]

## p27 and Tumor Progression

A hallmark of cancer is unrestricted cell growth and proliferation; a crucial genetic abnormality that occurs during tumor development is the loss of control at the $G_1$ to S transition, which is a period during which cells decide to either enter the cell cycle on mitogenic stimuli, or stay quiescent in response to antimitogenic or senescence signals. Hypophosphorylated pRb is pivotal in maintaining the quiescent state and controlling the $G_1$ to S checkpoint. Phosphorylation inactivates pRb, which allows a cell to enter the S phase. CDK family members, along with their corresponding cyclins expressed during the S phase, mediate the phosphorylation of several critical targets, including the Rb gene product, *pRb*. This CDK-mediated phosphorylation is inhibited by specific proteins called CDK inhibitors, or CDKIs, of which p27 is a family member.[11,12]

Most normal epithelial tissues, including breast, prostate, lung, and ovary, express high nuclear levels of the p27 protein. A variable loss of p27 has been observed in all human malignancies examined to date.[13] Even as a tumor suppressor, p27 does not fit the classic tumor suppressor paradigm in humans, as mutations in or the silencing of the

[1]Department of Oncology, Jinling Hospital, School of Medicine, Nanjing University, Nanjing, China.
[2]The Clinical School of Nanjing Medical University, Nanjing, China.

*Address correspondence to: Xiaoxiang Guan; Department of Oncology, Jinling Hospital, School of Medicine, Nanjing University; 305 Zhongshan East Road, Nanjing 210002, China*
*E-mail:* **xguan@nju.edu.cn**

*p27* gene in human tumors are extremely rare.[14] However, a loss of or decrease in p27 protein levels is frequently observed in many human cancers. Current studies have reported that, in addition to cytosolic mislocalization, p27 expression levels decrease during breast cancer development and progression. A number of studies have characterized p27 as an independent prognostic factor in breast cancer.[15–18]

p27 is regulated at both the expression level and subcellular localization.[12] The absence or reduction of p27 expression has been associated with the aggressive behavior of human malignancies, including breast, gastric, prostate, colon, and lung carcinomas.[19] The exact molecular mechanisms responsible for the down-regulation and mislocalization of p27 remain unknown. However, phosphorylation has a high correlation with the mislocalization of p27 to the cytoplasm.

### Phosphorylation of p27

Although transcriptional and noncovalent sequestration may pretranscriptionally regulate p27, the protein is primarily regulated post-transcriptionally at the level of both translation and stability. It has been suggested that two proteolytic pathways act in sequence during the cell cycle to control the abundance of p27. The first functions during the early and mid-$G_1$ phase, and is triggered by serum independently of Thr187 phosphorylation and CDK2. The second pathway works in the late $G_1$, S, and $G_2$ phases, and is dependent on both Thr187 phosphorylation and CDK2. The second pathway is well characterized and discussed in greater detail next, but the first pathway has not yet been completely elucidated. The p27 protein may also be degraded through a mitogen-activated protein kinase (MAPK)-dependent and CDK2-independent process.[20–22]

In addition to Thr187, there are three additional phosphorylation sites, including Ser10, Thr157, and Thr198, which are involved in cellular localization. The phosphorylation at Ser10 stabilizes the p27 protein in $G_1$ and accounts for 75% of p27 phosphorylation. p27 is exported to the cytoplasm in a KPC1/2-induced ubiquitin-dependent degradation manner. Based on this mechanism, p27 is phosphorylated at Ser10, which promotes CRM1-dependent binding and nuclear export. This pathway operates in $G_1$ cells or cells stimulated to re-enter the cell cycle from a quiescent state. Phosphorylation at Thr157 by protein kinase B/Akt impairs the nuclear import of p27, but does not affect its stability in breast cancer and other cell types.[23] Fujita et al. indicated that p27 phosphorylation at Thr198 by p90 ribosomal protein S6 kinases promotes cytoplasmic localization. Mutations in these phosphorylation sites may result in a more potent induction of apoptosis and the inhibition of cell growth for breast cancer gene therapy. The phosphorylation state of p27 is involved in signal transduction, apoptosis, and gene expression pathways.[24] However, the specific mechanisms involved in the phosphorylation regulation of p27 expression remain elusive, and a better understanding of how this pathway interacts with its signals in both the cytoplasm and nucleus is needed.

### Signal Transduction Pathway of p27

Signal transduction is the process by which an extracellular stimulus is converted to an intracellular signal that triggers a cascade or series of events, ultimately culminating in the production of an appropriate cellular response.[25] The mechanisms controlling p27 abundance range from translational control observed in quiescent cells to proteolytic mechanisms operating at specific phases of the cell cycle or in specific subcellular compartments, such as the cytoplasm or nucleus. The concentration of p27 is thought to be predominantly regulated by the ubiquitin-dependent proteolytic pathway. The degradation of p27 is triggered by its phosphorylation on Thr187 by the cyclin E-CDK2 complex. The phosphorylation of Thr187 is required for the binding of p27 to Skp2, which is the F-box protein component of an SCF ubiquitin ligase (E3) complex, and this interaction, in turn, results in the polyubiquitination and degradation of p27. The control of p27 protein levels is affected by ubiquitin-dependent degradation in a ubiquitin- and Skp2-independent manner at $G_1$ as well as by JAB1-dependent degradation. The effect of p27 on the cell cycle is predominantly regulated by its stability and subcellular localization.[20–22,26]

Ubiquitin-proteasome proteolysis is the major post-transcriptional pathway that is utilized for the regulation of p27. The removal of p27 CDK inhibitory activity in $G_1$, thus, appears to occur in at least three phases in this model system: sequestration in cyclin D-CDK complexes in early $G_1$, export/cytoplasmic degradation in mid-$G_1$, and Skp2-dependent degradation in the nuclear compartment beginning late in the $G_1$ phase. The phosphorylation of the p27 protein on Thr187 by CDK2 prepares the p27 protein for binding to the ubiquitin ligase SCF, which leads to 26S proteasome degradation. It has been proved that the Ras-MAPK pathway is the most likely signaling pathway involved in this process, occupying a central position in the network of interactive signaling cascades. The MAP kinases p42MAPK, or ERK1, and p44MAPK, or ERK2, are regulated as the central components of the growth-promoting signaling pathways. A dominant negative mutant of Grb2 and inhibitors of ERK1/2 can rescue the decrease in the p27 protein. In addition, ERK1/2 has been reported to regulate Thr160 phosphorylation of CDK2, which is required for activation of the kinase. Two types of dominant negative Ras proteins have been shown to block p27 deregulation due to increased CDK2-cyclinE activity. It has been observed that ERK2 can directly phosphorylate the p27 protein on Ser10. Overexpression of endogenous and exogenous Ras-MAPK decreases the half-life of p27 and reduces CDK2 activity. The inhibition of Ras-MAPK leads to an accumulation of p27 and decreased CDK2 activity. It is apparent that Ras-MAPK plays a role in the regulation of p27 protein levels.[25,27]

Previous studies have shown that PI3K-AKT regulates the expression of the p27 protein transcriptionally through forkhead transcription factors. The forkhead transcription factors activate the *p27* gene, which leads to increased protein levels. Several studies have indicated that the inhibition of PI3K either by chemical inhibitors or by a dominant negative mutant of p85, which is the regulatory subunit of PI3K, can increase p27 and decrease p27-CDK2 activity. The expression of a dominant negative AKT can also increase p27 protein expression. In addition to the transcriptional suppression of *p27* achieved by inhibiting forkhead transcription factors, the PI3K pathway is also able to post-transcriptionally regulate the localization and level of p27 through direct threonine phosphorylation of p27 by AKT. Several groups have demonstrated that AKT phosphorylates p27 at Thr157

*in vitro* and *in vivo*. Thr157 is in the nuclear localization motif of p27, and its phosphorylation causes p27 to exit the nucleus, which rescues CDK2 from both p27-induced inhibition and proteasomal degradation. In addition, AKT has been reported to be able to phosphorylate p27 at Thr198, which also causes the protein to localize to the cytoplasm. Clinically, the THR2-targeted antibody trastuzumab affects the phosphorylation of Thr157 and Thr198, which regulates the expression of p27 in the cytosol of breast cancer cells.[28]

As just described, the phosphorylation of Thr187 helps degrade p27, but Ser10 phosphorylation increases its stability. Nuclear protein human kinase-interacting stathmin (hKIS) phosphorylates Ser10; this phosphorylation, however, does not stabilize p27, but instead promotes its export to the cytoplasm. Ser10 can be phosphorylated by various kinases, which determines the phosphorylation outcome during $G_0$ and $G_1$. Ser10 phosphorylation by the mitogen-induced kinase hKIS leads to the translocation of the protein from the nucleus to the cytosol, where p27 cannot inhibit CDK2 and is also susceptible to proteolysis. hKIS overexpression overcomes the growth arrest induced by p27, whereas the depletion of hKIS with siRNA inhibits Ser10 phosphorylation and enhances growth arrest.[29,30]

Other known molecules are involved in this specific signal transduction pathway as well, including TGF-$\beta$, IL-6, and STAT3. The inhibition at any level of this signal transduction pathway or the modification of the phosphorylation state of these molecules can regulate p27 function. Targeted drugs that interfere with the signal transduction cascade in blocking tumor cell proliferation seem promising in cancer therapy.[31] However, in order to establish screens for identifying the key inhibitors of p27 in tumor cells and to understand the exact mechanisms involved in the mislocalization and down-regulation of expression, a profile of p27 interacting partners should first be elucidated.

### Interacting Protein Profiling of p27

Cellular proliferation can occur at different rates and is dependent on extracellular factors and genetic profiles, which determine the differentiation status and the eventual transformation grade. The proliferation rate is regulated by the proteins that are involved in cell-cycle control, specifically cyclins and their associated kinases. The fluctuating activities of several Cdk/Cyclin complexes establish the progression of the cell cycle through each phase ($G_1$, S, $G_2$, and M). The composition and regulation of Cdks vary according to cell type and environment.[32] In addition to cyclins and their associated kinases, the CDKIs are the third group of proteins involved in cell-cycle progression. Among them, p27 plays a crucial role in linking extracellular growth-regulatory signals to the progression or exit from the cell cycle. CDK2 is the predominant kinase that allows progression through the $G_1$/S phase and subsequent replication events.[2] p27 is a CDKI that binds to CDK2 to prevent its premature activation.[6] The protein can also directly bind to a CDK-activating kinase through its C-terminal domain.[31]

p27 has been reported to interact with a number of intracellular and extracellular molecules, including CRM1, Jab1, SKP2, Ras, Spy1, and TGF-$\alpha$.[33–38] The overexpression of Jab1/CSN5 elicits nuclear export/degradation of p27, suggesting that the relocalization of these molecules is required before degradation. Recent studies have further characterized the mechanisms underlying p27 export. Jab1/CSN5 promotes the export and degradation of p27 through a CRM1-dependent mechanism, possibly involving interactions of p27 with the adaptor protein Grb2 in the cytoplasm. The export of p27 to the cytoplasm after growth factor stimulation may also inhibit Grb2/SOS interactions and serve to limit Ras effector functions.

Several additional molecules are specific for breast cancer, such as the estrogen receptor, progesterone receptor, and ErbB-2. Gompel et al.[39] showed that E2 increases the level of the various cyclins involved in cell-cycle progression and decreases the cyclin kinase inhibitors p27. Progesterone up-regulates *p27* expression, and induction is required for the growth-inhibitory action of progesterone.[40,41] Research also has shown that a combined expression of high/low p27(kip1) and low/high Ki-67 values showed 100% specificity and sensitivity in predicting long-term versus short-term survivors.[42] Moreover, ErbB-2 induces mammary tumors. Experiments conducted on cultured cells have shown that p27 can inhibit ErbB-2 signaling. It has also been suggested that p27 may be the downstream target of HER-2/neu oncogenic signals in promoting tumor development, and p27 is a potential target for HER-2/neu-associated tumors.[43] Furthermore, our meta-analysis indicated that p27 is an independent prognostic factor for breast cancer.[44] With continued efforts in cancer research, new therapeutic molecules will undoubtedly be discovered; however, only full characterization and assessment will elucidate their potential clinical utility.

### Conclusions

In conclusion, we hypothesize that crosstalk between p27 and other molecules in this signaling pathway plays an indispensable role in cell proliferation and differentiation. The imbalance of this crosstalk may lead to the malignancy of breast cancer. Furthermore, understanding the molecular profile of p27 could lead us into a contemporary era of tailored and individualized breast cancer treatment.

### Acknowledgments

This study was supported by the National Natural Science Foundation Grants (Nos. 81141094 and 30870962, to X.G.), the Natural Science Foundation of Jiangsu Province (BK2011656, to X G), and the General Hospital of Nanjing Military Area Grants No. (2009M013, to X.G.).

### Disclosure Statement

No competing financial interests exist.

### References

1. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000;100:57.
2. Vidal A, Koff A. Cell-cycle inhibitors: Three families united by a common cause. Gene 2000;247:1.
3. Guan X, Chen L, Wang J, et al. Mutations of phosphorylation sites Ser10 and Thr187 of $p27^{Kip1}$ abolish cytoplasmic redistribution but do not abrogate $G_0/_1$ phase arrest in the $HepG_2$ cell line. Biochem Biophys Res Commu 2006;347:601.
4. Slingerland J, Pagano M. Regulation of the cdk Inhibitor p27 and Its deregulation in Cancer. Cell Physiol 2000;183:10.

5. Chen F, Kim E, Wang C, et al. Ciglitazone-induced p27 gene transcriptional activity is mediated through Sp1 and is negatively regulated by the MAPK signaling pathway. Cell Signal 2005;17:1572.
6. Zhang Q, Tian L, Abdel Mansouri A, et al. Inducible expression of a degradation-resistant form of $p27^{Kip1}$ causes growth arrest and apoptosis in breast cancer cells. FEBS Lett 2005;579:3932.
7. Nguyen DX, Massagué J. Genetic determinants of cancer metastasis. Nat Rev Genet 2007;8:341.
8. Blain SW, Scher HI, Cordon CC. p27 as a target for tumor therapeutics. Cancer Cell 2003;3:111.
9. Katner AL, Hoang QB, Gootam P, et al. Induction of cell cycle arrest and apoptosis in human prostate carcinoma cells by a recombinant adenovirus expressing p27(Kip1). Prostate 2002;53:77.
10. Loda M, Cukor B, Tam SW, et al. Increased proteasome-dependent degradation of the cyclin-dependent kinase inhibitor p27 in aggressive colorectal carcinomas. Nat Med 1997;3:231.
11. Guan X, Chen L, Wang J. Protein profiling: A possible molecular mechanism to mislocalization and down-expression of $p27^{Kip1}$ in tumor cells. Med Hypotheses 2007;69:508.
12. Berthet C, Kaldis P. Cell-specific responses to loss of cyclin-dependent kinases. Oncogene 2007;7:1.
13. Viglietto G, Motti ML, Fusco A. Understanding p27(kip1) deregulation in cancer: Down-regulation or mislocalization. Cell Cycle 2001;1:394.
14. Yang ES, Burnstein KL. Deregulation of p27 by oncogenic signaling and its prognostic significance in breast cancer. Breast Cancer Res 2004;6:13.
15. Kouvaraki M, Vassilis G, Gorgoulis, et al. High expression levels of p27 correlate with lymph node status in a subset of advanced invasive breast carcinomas. Cancer 2002;94:2454.
16. Blain SW, Massague J. Breast cancer banishes $p27^{Kip1}$ from nucleus. Nat Med 2002;8:1076.
17. Barnes A, Pinder SE, Bell JA, et al. Expression of $p27^{Kip1}$ in breast cancer and its prognostic significance. J Pathol 2003; 201:451.
18. De Paola F, Vecci AM, Granato AM, et al. p27/kip1 expression in normal epithelium, benign and neoplastic breast lesions. Pathol J Pathol 2002;196:26.
19. Lacy ER, Wang YF, Post J, et al. Molecular basis for the specificity of p27 toward cyclindependent kinases that regulate cell division. J Mol Biol 2005;349:764.
20. Sherr CJ, Roberts JM. CDK inhibitors: Positive and negative regulators of $G_1$-phase progression. Genes Develop 1999;13: 1501.
21. Philip SJ, Payne SR, Kemp CJ. $p27^{Kip1}$: Regulation and function of a haloinsufficient tumor supresssor and its misregulation in cancer. Exp Cell Res 2001;264:148.
22. Pagano M, Tam SW, Theodoras AM, et al. Role of the ubiquitin-proteasome pathway in regulating abundance of the cyclin-dependent kinase inhibitor p27. Science 1995;269:682.
23. Shin I, Yakes FM, Rojo F, et al. PKB/Akt mediates cell-cycle progression by phosphorylation of p27(Kip1) at threonine 157 and modulation of its cellular localization. Nat Med 2002;8:1145.
24. Fujita N, Sato S, Tsuruo T. Phosphorylation of p27Kip1 at threonine 198 by p90 ribosomal protein S6 kinases promotes its binding to 14-3-3 and cytoplasmic localization. J Biol Chem 2003;78:49254.
25. Emily F, Andy S, Lisa PM, et al. Integration of progesterone receptor mediated rapid signaling and nuclear actions in breast cancer cell models: Role of mitogen-activated protein kinases and cell cycle regulators. Steroids 2005;70:418.
26. Rodier G, Montagnoli A, Di ML, et al. p27 cytoplasmic localization is regulated by phosphorylation on Ser10 and is not a prerequisite for its proteolysis. EMBO J 2001;20:6672.
27. Meloche S, Pouysse J. The ERK1/2 mitogen-activated protein kinase pathway as a master regulator of the G1- to S-phase transition. Oncogene 2007;26:3227.
28. Liang J, Zubovitz J, Petrocelli T, et al. PKB/Akt phosphorylates p27, impairs nuclear import of p27and opposes p27-mediated G1 arrest. Nat Med 2002;8:1153.
29. Viglietto G, Motti ML, Bruni P, et al. Cytoplasmic relocalization and inhibition of the cyclindependent kinase inhibitor p27(Kip1) by PKB/Akt-mediated phosphorylation in breast cancer. Nat Med 2002;8:1136.
30. Ishida N, Kitagawa M, Hatakeyama M, et, al. Phosphorylation at serine 10, a major phosphorylation site of p27(Kip1), increases its protein stability. J Biol Chem 2000; 275:25146.
31. James H, Lee RJ, Russell RG. ErbB-2-induced mammary tumor growth: The role of cyclin D1 and $p27^{Kip1}$. Biochem Pharma 2002;64:827.
32. Sgambato A, Cittadini A, Faraglia B, et al. Multiple Functions of p27Kip1 and Its Alterations in Tumor Cells. Cell Physiol 2000;183:18.
33. Ishida N, Hara T, Kamura T, et al. Phosphorylation of p27 Kip1on serine 10 is required for it s binding to CRM1 and nuclear export. J Biol Chem 2002;277:14355.
34. Fukumoto A, Ikeda N, Sho M,*et al*. Prognostic significance of localized p27Kip1 and potential role of Jab1/CSN5 in pancreatic cancer. Oncol Rep 2004;11:277.
35. James SF, Romaine IF, Noriko I, et al. Estrogens down-regulate p27Kip1 in breast cancer cells through Skp2 and through nuclear export mediated by the ERK pathway. Biol Chem 2003;278:41355.
36. Pruitt K, Der CJ. Ras and Rho regulation of the cell cycle and oncogenesis. Cancer Lett 2001;171:100.
37. Porter LA, Kong-Beltran M, Donoghue DJ. Spy1 interacts with p27Kip1 to allow G1/S progression. Mol Biol Cell 2003;14:3664.
38. Zhang F, Monkkonen M, Roth S. TGF-beta induced G(1) cell cycle arrest requires the activity of the proteasome path2 way. Transformoring growth factor. Exp Cell Res 2002; 281:190.
39. Gompel A, Chaouat M, Hugol D, et al. Steroidal hormones and proliferation, differentiation and apoptosis in breast cells. Maturitas 2004;49:16.
40. Gizard F, Robillard R, Gervois P, et al. Progesterone inhibits human breast cancer cell growth through transcriptional up-regulation of the cyclin-dependent kinase inhibitor p27Kip1 gene. FEBS Lett 2005;579:5535.
41. Way TD, Kaob MC, Lin JK. Degradation of $HER_2$/neu by apigenin induces apoptosis through cytochrome c release and caspase-3 activation in HER2/neu-overexpressing breast cancer cells. FEBS Lett 2005;579:145.
42. Bongiovanni M, Cassoni P, De Giuli P, et al. p27(kip1) immunoreactivity correlates with long-term survival in pleural malignant mesothelioma. Cancer 2001;92:1245.
43. Lisa N. Correlation of p27 protein expression with HER-2/neu expression in breast cancer. Mol Carcinog 2001; 30:169.
44. Guan X, Wang Y, Xie R, et al. p27 as a prognostic factor in breast cancer: A systematic review and meta-analysis. J Cell Mol Med 2010;14:944.