PERSPECTIVE

Duality of p27^{Kip1} function in tumorigenesis

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In this issue of *Genes & Development*, Besson et al. (2007) report phenotypic characteristics of a novel knock-in mouse strain expressing a mutant allele of the cell cycle inhibitor p27^{Kip1} that has both common and unique features when compared with a null allele. The new studies provide the first direct in vivo evidence that in addition to its role as a tumor suppressor, p27^{Kip1} also functions as an oncogene. The work also suggests that p27^{Kip1} oncogenic activity leads to aberrant stem and progenitor cell expansion in the lung and retina, respectively. Thus, p27^{Kip1}'s new "dark side" may serve an oncogenic function that operates in less specialized cell types to influence tumorigenesis.

The 'good' side of p27^{Kip1} function

The progression of cells through the cell cycle is driven by enzymatic complexes composed of cyclins and their catalytic partners, cyclin-dependent kinases (CDKs) (Sherr and Roberts 1999). When p27^{Kip1} was originally cloned, it was thought to act as an inhibitor of cyclin-CDK complexes (Polyak et al. 1994a,b; Toyoshima and Hunter 1994). However, it soon became obvious that the picture was not so simple, as evidence for an additional function for p27^{Kip1} was provided by the observations that this cell cycle inhibitor also serves as an essential assembly factor for cyclin D-CDK4 and cyclin D-CDK6 complexes (LaBaer et al. 1997; Cheng et al. 1999). More recent studies revealed that p27^{Kip1} plays additional, cell cycle-independent roles. The full repertoire of these functions is only beginning to be appreciated (Fig. 1). So far, these novel functions include regulation of the actin cytoskeleton and cell migration through modulation of RhoA activity (McAllister et al. 2003; Besson et al. 2004; Wu et al. 2006), as well as promoting neuronal differentiation by stabilizing Neurogenin-2 protein (Nguyen et al. 2006).

p27^{Kip1} is a tumor suppressor. Indeed, reduced p27^{Kip1} levels in primary cancers are highly correlated with de-

creased patient survival (Slingerland and Pagano 2000). Germline mutation of p27^{Kip1} was also recently described in a subset of MEN (Multiple Endocrine Neoplasia) syndrome patients in humans, as well as in rats with the related MENX syndrome (Pellegata et al. 2006). p27^{Kip1}-null mice develop organomegaly and pituitary adenomas (Fero et al. 1996; Kiyokawa et al. 1996; Nakayama et al. 1996). The most simplistic explanation of the phenotypes was that in p27^{Kip1}^{-/-} mice, the deregulated CDK2 kinase was responsible for these symptoms. However, recent observations that ablation of CDK2 in p27^{Kip1}-null background did not mitigate the phenotypes of p27^{Kip1} deficiency clearly shows that p27^{Kip1} can act independently of CDK2, likely in part by hyperactivating CDK1 (Aleem et al. 2005; Martin et al. 2005).

p27^{Kip1} as an oncogene

A number of perplexing observations regarding p27^{Kip1} activity in murine and human patients appear to contradict the expected role of a tumor suppressor. Although human cancers often express very low levels of p27^{Kip1}, the homozygous loss of p27^{Kip1} is extremely rare. p27^{Kip1}^{-/-} animals were more susceptible to mammary and prostate tumors than p27^{Kip1}-null mice, suggesting an active contribution of the intact wild-type p27^{Kip1} allele in tumorigenesis (Muraoka et al. 2002; Gao et al. 2004). Lastly, a correlation between poor prognosis and cytoplasmic localization of p27^{Kip1} in human tumors suggests an active tumor-promoting function of p27^{Kip1} in the cytoplasm (Slingerland and Pagano 2000). These findings have led to the hypothesis that p27^{Kip1} has an additional, oncogenic function.

The study by Besson et al. (2007) tested the contribution of CDK-independent functions of p27^{Kip1} by analyzing knock-in mice expressing mutant p27^{Kip1} (p27^{CK}) that is unable to inhibit cyclin-CDK complexes. One might expect that the phenotype of the homozygous p27^{CK/CK} mice would resemble that of p27^{Kip1}-null animals. This is true to a certain extent, as p27^{CK/CK} mice displayed organomegaly and spontaneous pituitary tumor development, like the original p27^{Kip1}-null mice. Very unexpectedly, however, Besson et al. (2007) found

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Figure 1. Model of the roles of p27^{Kip1}. (Left) p27^{Kip1} affects cell cycle progression via interaction with cyclin–CDK complexes and has also been shown to have cell cycle-independent roles such as regulating RhoA activity. (Right) The work of Besson et al. (2007) suggests that p27^{Kip1} activity related to cell cycle regulation may be more relevant to its role as a tumor suppressor, whereas p27^{Kip1} also has cyclin–CDK-independent oncogenic activity. Whether the tumor-suppressing or oncogenic activity of p27^{Kip1} can be assigned strictly to the pathways depicted or compartmentalized based on location in the nucleus or cytoplasm as shown still remains to be determined in detail.

that p27^{CK/CK} mice also developed a whole range of hyperplastic and neoplastic lesions, including lung adenocarcinomas. This truly stunning observation indicates that the loss of CDK-inhibitory activity unmasks evidence for the oncogenic function of p27^{Kip1}. A similar phenotype arose in p27^{CK/+} mice, although at a longer latency. Importantly, no loss of heterozygosity was observed in tumors from these mice, adding weight to the hypothesis that this work uncovers an oncogenic role for p27^{Kip1}. One potential caveat of this work that should also be noted is that, in some contexts, the levels of p27^{Kip1} appear elevated compared with wild-type tissue. Thus, it cannot be formally ruled out that some aspects of the phenotype of p27^{CK} mice are not due to subtle overexpression.

At present, we can only speculate as to the mechanistic basis for p27^{Kip1Kip1} oncogenicity. In human cancer cells, cytoplasmic localization of p27^{Kip1Kip1} was shown to correlate with high tumor grade and poor prognosis (Slingerland and Pagano 2000). Intriguingly, the p27^{CK} mutant also localizes to the cytoplasm, suggesting that a cytoplasmic function might mediate the oncogenic effect (Fig. 1). However, the p27^{CK} protein is not exclusively located in the cytoplasm; future studies that include restriction of p27^{Kip1} to particular cellular compartments will likely be key for understanding the mechanism of p27^{Kip1} oncogenic function. Of note, p21^{Cip1}, another cyclin-dependent kinase inhibitor, also exhibits cytoplasmic functions that are likely to be oncogenic (Coqueret 2003), although the formal demonstration for such activity in vivo remains to be seen. Therefore, the work of Besson et al. (2007) may provide the first evidence for similar cytoplasmic-based oncogenic activity of multiple CDK inhibitors. An extensive discussion of the potential effect of p27^{Kip1} oncogenic activity via RhoA was provided by Besson et al. (2007), and since the phenotypes observed in p27^{CK} mice in this

study were highlighted by their comparison with p27^{Kip1}-null animals, future comparisons with other p27^{Kip1} point mutants that selectively inhibit RhoA pathway alterations, for example, could clarify the CDK-independent functions of p27^{Kip1} in tumorigenesis.

p27^{Kip1} regulation of stem and progenitor cells

Coupled with oncogenic activity, Besson et al. (2007) reported an increased pool of bronchioalveolar stem cells (BASCs) in p27^{CK} mice, suggesting that p27^{Kip1} acts within lung stem cells. Cells with both the alveolar marker SPC and the bronchiolar cell marker CC10, which we previously defined as BASCs (Kim et al. 2005), were more abundant in p27^{CK} lungs compared with wild-type lungs. It remains to be confirmed whether BASCs from p27^{CK} mice have altered stem cell characteristics; ex vivo analysis of the effect of p27^{Kip1} activity on BASC self-renewal and differentiation will be informative. Importantly, it appears that the bronchiolar Clara cells, in addition to BASCs, are also stimulated to enter the cell cycle in the presence of the p27^{CK} allele. Therefore, it is not apparent whether p27^{CK} exerts its effects specifically on BASCs, or rather on multiple types of lung epithelial cells. Adding to the likely complex nature of p27^{CK} effects in lung, these mutant mice also developed desquamative interstitial pneumonitis, characterized by an influx of macrophages, neutrophils, and infiltrating lymphocytes that was often difficult to distinguish from tumorigenic lesions. Given this dramatic inflammatory response, it will be of note to determine whether the observed expansion of BASCs or other lung epithelial cells is attributable to signals from inflammatory cells or a cell-autonomous effect.

It is possible that the bulk of p27^{Kip1} activity, both cyclin-dependent and putative oncogenic activity, has its readout in progenitor cell populations, rather than specifically in stem cells. Pax6-, Chx10-, and BrdU-positive retinal cells were present in increased numbers in the p27^{CK} retina, suggesting an enhanced progenitor phenotype. Besson et al. (2007) note several other tissue-specific progenitor cell types, rather than stem cells, which may be affected by p27^{Kip1} function; committed progenitors in the hematopoietic and glial lineages were increased in p27^{Kip1} mice, whereas hematopoietic stem cells were not (Cheng and Scadden 2002). In fact, members of both the INK4 and CIP/KIP families of CDK inhibitors have long been implicated in the coordination of cell cycle withdrawal and initiation of differentiation in various cell types. Levels of p27^{Kip1} in differentiated, nonproliferating tissues are often increased relative to their more immature counterparts (De Clercq and Inze 2006), as has been observed in intestinal epithelial stem/progenitor cells (Quaroni et al. 2000), chondrocytes (Yeh et al. 2007), neuronal progenitors (Nguyen et al. 2006), as well as in ES cells (Egozi et al. 2007). In addition, p27^{Kip1} frequently exhibits reduced expression in more poorly differentiated, high-grade tumors (Tsihlias et al. 1999). Interestingly, the ability of p27^{Kip1} to promote myogenic and neuronal differentiation in *Xenopus*, via regulation

Figure 2. Cartoon representation of effects on lung stem cell number and tumorigenesis. (*Top*) In the normal lung, BASCs are present in low abundance in the bronchioalveolar niche, where it is hypothesized they can differentiate into Clara cells that line the bronchioles or the alveolar epithelial type 1 and 2 cells (AT1/2) that form the lung alveolar region. (*Bottom*) Recent findings indicate that abrogation of a number of pathways similarly leads to an increase in BASC numbers and enhanced lung cancer susceptibility. The route by which BASC expansion might lead to lung cancer formation and the direct impact of these mutations on stem cell function are open questions.

of the transcription factors MyoD and neurogenin, respectively, is CDK independent (Vernon and Philpott 2003; Vernon et al. 2003). The role of p27^{Kip1} in specific cell subsets within given tissues will certainly be worked out with further studies.

As for the oncogenic activity of p27^{Kip1}, its precise molecular mechanisms in stem or progenitor cell regulation are also largely unknown. Interestingly, RhoA inhibition in hematopoietic stem and progenitor cells lead to increased engraftment and self-renewal (Ghiaur et al. 2006). As Besson et al. (2007) hypothesize that RhoA deregulation is involved in the mechanisms of p27^{CK} phenotype, the similar phenotypes of p27^{Kip1} and RhoA abrogation in stem or progenitor cells are intriguing. No apparent migratory activity of BASCs was noted in p27^{CK} mice as might be expected for RhoA misregulation, but proliferative retinal progenitors were in ectopic locations, possibly suggesting a tissue-specific effect on cell migration and distinct mechanisms downstream from p27^{Kip1} in diverse tissues.

Using cell cycle inhibitor analysis to uncover the origins of cancer

The lung phenotype reported by Besson et al. (2007), together with recent reports analyzing several other genetic pathways, further support previous studies that expansion of an adult stem cell population in the lung leads to the development of non-small-cell lung cancer.

p27^{CK}, p38MAPK deficiency (Ventura et al. 2007), and p18^{Ink4c} deficiency (Pei et al. 2007) all appear to increase the number of BASCs and enhance susceptibility to lung tumorigenesis (along with other unknown components or when analyzed directly with activation of oncogenic K-ras) (Fig. 2). The abundance of proliferative retinal progenitors in p27^{CK} mice does not lead to retinoblastomas, and rather seems to activate an apoptotic response. The onset of lung tumorigenesis in p18^{-/-} and p27^{CK} mice occurs in aged adults, and even though BASC expansion in mutant lungs is reportedly widespread, these animals only develop one or two lung tumors. Thus, expansion of stem and progenitor cells is clearly not sufficient for the development of frank carcinomas in the lung or retinal tumors, and other genetic or epigenetic events likely cooperate with, for example, oncogenic p27^{Kip1}, to allow for tumor formation. Again, as above, it is not yet clear that these pathways only affect stem or progenitor cells, and so future studies allowing cell-type-specific genetic manipulation will be needed to truly determine which cell types give rise to cancer in the lung and other organs.

Clearly, more work is needed to elucidate the exact molecular oncogenic function of p27^{Kip1}. Studies to determine whether or not p27^{Kip1} exerts such functions on particular populations of cells and particular tissues will also likely provide important mechanistic insight on the origins of cancer and the regulatory pathways for stem and progenitor cell populations. Although we are only beginning to appreciate all cancer-relevant functions of p27^{Kip1}, the work of Besson et al. (2007) is a big step in the direction of getting to know the two sides of p27^{Kip1}.

References

- Aleem, E., Kiyokawa, H., and Kaldis, P. 2005. Cdc2-cyclin E complexes regulate the G1/S phase transition. *Nat. Cell Biol.* 7: 831–836.
- Besson, A., Gurian-West, M., Schmidt, A., Hall, A., and Roberts, J.M. 2004. p27^{Kip1} modulates cell migration through the regulation of RhoA activation. *Genes & Dev.* 18: 862–876.
- Besson, A., Hwang, H.C., Cicero, S., Donovan, S.L., Gurian-West, M., Johnson, D., Clurman, B.E., Dyer, M.A., and Roberts, J.M. 2007. Discovery of an oncogenic activity in p27^{Kip1} that causes stem cell expansion and a multiple tumor phenotype. *Genes & Dev.* (this issue), doi: 10.1101/gad.1556607.
- Cheng, T. and Scadden, D.T. 2002. Cell cycle entry of hematopoietic stem and progenitor cells controlled by distinct cyclin-dependent kinase inhibitors. *Int. J. Hematol.* 75: 460–465.
- Cheng, M., Olivier, P., Diehl, J.A., Fero, M., Roussel, M.F., Roberts, J.M., and Sherr, C.J. 1999. The p21(Cip1) and p27(Kip1) CDK 'inhibitors' are essential activators of cyclin D-dependent kinases in murine fibroblasts. *EMBO J.* 18: 1571–1583.
- Coqueret, O. 2003. New roles for p21 and p27^{Kip1} cell-cycle inhibitors: A function for each cell compartment? *Trends Cell Biol.* 13: 65–70.
- De Clercq, A. and Inze, D. 2006. Cyclin-dependent kinase inhibitors in yeast, animals, and plants: A functional comparison. *Crit. Rev. Biochem. Mol. Biol.* 41: 293–313.
- Egozi, D., Shapira, M., Paor, G., Ben-Izhak, O., Skorecki, K., and Herskko, D.D. 2007. Regulation of the cell cycle inhibitor p27 and its ubiquitin ligase Skp2 in differentiation of human

- embryonic stem cells. *Faseb J.* doi: 10.1096/fj.06-7758com.
- Fero, M.L., Rivkin, M., Tasch, M., Porter, P., Carow, C.E., Firpo, E., Polyak, K., Tsai, L.H., Broudy, V., Perlmutter, R.M., et al. 1996. A syndrome of multiorgan hyperplasia with features of gigantism, tumorigenesis, and female sterility in p27^{Kip1}(Kip1)-deficient mice. *Cell* **85**: 733–744.
- Gao, H., Ouyang, X., Banach-Petrosky, W., Borowsky, A.D., Lin, Y., Kim, M., Lee, H., Shih, W.J., Cardiff, R.D., Shen, M.M., et al. 2004. A critical role for p27^{Kip1}kip1 gene dosage in a mouse model of prostate carcinogenesis. *Proc. Natl. Acad. Sci.* **101**: 17204–17209.
- Ghiaur, G., Lee, A., Bailey, J., Cancelas, J.A., Zheng, Y., and Williams, D.A. 2006. Inhibition of RhoA GTPase activity enhances hematopoietic stem and progenitor cell proliferation and engraftment. *Blood* **108**: 2087–2094.
- Kim, C.F., Jackson, E.L., Woolfenden, A.E., Lawrence, S., Babar, I., Vogel, S., Crowley, D., Bronson, R.T., and Jacks, T. 2005. Identification of bronchioalveolar stem cells in normal lung and lung cancer. *Cell* **121**: 823–835.
- Kiyokawa, H., Kineman, R.D., Manova-Todorova, K.O., Soares, V.C., Hoffman, E.S., Ono, M., Khanam, D., Hayday, A.C., Frohman, L.A., and Koff, A. 1996. Enhanced growth of mice lacking the cyclin-dependent kinase inhibitor function of p27(Kip1). *Cell* **85**: 721–732.
- LaBaer, J., Garrett, M.D., Stevenson, L.F., Slingerland, J.M., Sandhu, C., Chou, H.S., Fattaey, A., and Harlow, E. 1997. New functional activities for the p21 family of CDK inhibitors. *Genes & Dev.* **11**: 847–862.
- Martin, A., Odajima, J., Hunt, S.L., Dubus, P., Ortega, S., Malumbres, M., and Barbacid, M. 2005. Cdk2 is dispensable for cell cycle inhibition and tumor suppression mediated by p27(Kip1) and p21(Cip1). *Cancer Cell* **7**: 591–598.
- McAllister, S.S., Becker-Hapak, M., Pintucci, G., Pagano, M., and Dowdy, S.F. 2003. Novel p27^{Kip1}(kip1) C-terminal scatter domain mediates Rac-dependent cell migration independent of cell cycle arrest functions. *Mol. Cell. Biol.* **23**: 216–228.
- Muraoka, R.S., Lenferink, A.E., Law, B., Hamilton, E., Brantley, D.M., Roebuck, L.R., and Arteaga, C.L. 2002. ErbB2/Neu-induced, cyclin D1-dependent transformation is accelerated in p27^{Kip1}-haploinsufficient mammary epithelial cells but impaired in p27^{Kip1}-null cells. *Mol. Cell. Biol.* **22**: 2204–2219.
- Nakayama, K., Ishida, N., Shirane, M., Inomata, A., Inoue, T., Shishido, N., Horii, I., Loh, D.Y., and Nakayama, K. 1996. Mice lacking p27^{Kip1}(Kip1) display increased body size, multiple organ hyperplasia, retinal dysplasia, and pituitary tumors. *Cell* **85**: 707–720.
- Nguyen, L., Besson, A., Heng, J.I., Schuurmans, C., Teboul, L., Parras, C., Philpott, A., Roberts, J.M., and Guillemot, F. 2006. p27kip1 independently promotes neuronal differentiation and migration in the cerebral cortex. *Genes & Dev.* **20**: 1511–1524.
- Pei, X.H., Bai, F., Smith, M.D., and Xiong, Y. 2007. p18Ink4c collaborates with Men1 to constrain lung stem cell expansion and suppress non-small-cell lung cancers. *Cancer Res.* **67**: 3162–3170.
- Pellegata, N.S., Quintanilla-Martinez, L., Siggelkow, H., Samson, E., Bink, K., Hofler, H., Fend, F., Graw, J., and Atkinson, M.J. 2006. Germ-line mutations in p27^{Kip1}Kip1 cause a multiple endocrine neoplasia syndrome in rats and humans. *Proc. Natl. Acad. Sci.* **103**: 15558–15563.
- Polyak, K., Kato, J.Y., Solomon, M.J., Sherr, C.J., Massague, J., Roberts, J.M., and Koff, A. 1994a. p27Kip1, a cyclin–Cdk inhibitor, links transforming growth factor- β and contact inhibition to cell cycle arrest. *Genes & Dev.* **8**: 9–22.
- Polyak, K., Lee, M.H., Erdjument-Bromage, H., Koff, A., Roberts, J.M., Tempst, P., and Massague, J. 1994b. Cloning of p27Kip1, a cyclin-dependent kinase inhibitor and a potential mediator of extracellular antimitogenic signals. *Cell* **78**: 59–66.
- Quaroni, A., Tian, J.Q., Seth, P., and Ap Rhys, C. 2000. p27(Kip1) is an inducer of intestinal epithelial cell differentiation. *Am. J. Physiol. Cell Physiol.* **279**: C1045–C1057.
- Sherr, C.J. and Roberts, J.M. 1999. CDK inhibitors: Positive and negative regulators of G1-phase progression. *Genes & Dev.* **13**: 1501–1512.
- Slingerland, J. and Pagano, M. 2000. Regulation of the cdk inhibitor p27^{Kip1} and its deregulation in cancer. *J. Cell. Physiol.* **183**: 10–17.
- Toyoshima, H. and Hunter, T. 1994. p27, a novel inhibitor of G1 cyclin–Cdk protein kinase activity, is related to p21. *Cell* **78**: 67–74.
- Tsihlias, J., Kapusta, L., and Slingerland, J. 1999. The prognostic significance of altered cyclin-dependent kinase inhibitors in human cancer. *Annu. Rev. Med.* **50**: 401–423.
- Ventura, J.J., Tenbaum, S., Perdiguero, E., Huth, M., Guerra, C., Barbacid, M., Pasparakis, M., and Nebreda, A.R. 2007. p38 α MAP kinase is essential in lung stem and progenitor cell proliferation and differentiation. *Nat. Genet.* **39**: 750–758.
- Vernon, A.E. and Philpott, A. 2003. A single cdk inhibitor, p27Xic1, functions beyond cell cycle regulation to promote muscle differentiation in *Xenopus*. *Development* **130**: 71–83.
- Vernon, A.E., Devine, C., and Philpott, A. 2003. The cdk inhibitor p27^{Kip1}Xic1 is required for differentiation of primary neurons in *Xenopus*. *Development* **130**: 85–92.
- Wu, F.Y., Wang, S.E., Sanders, M.E., Shin, I., Rojo, F., Baselga, J., and Arteaga, C.L. 2006. Reduction of cytosolic p27^{Kip1}(Kip1) inhibits cancer cell motility, survival, and tumorigenicity. *Cancer Res.* **66**: 2162–2172.
- Yeh, N., Miller, J.P., Gaur, T., Capellini, T.D., Nikolich-Zugich, J., de la Hoz, C., Selleri, L., Bromage, T.G., van Wijnen, A.J., Stein, G.S., et al. 2007. Cooperation between p27^{Kip1} and p107 during endochondral ossification suggests a genetic pathway controlled by p27^{Kip1} and p130. *Mol. Cell. Biol.* doi: 10.1128/MCB.02431-06.

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