Adams, N.C., Lin, H.C., Holash, J., et al. (2004). Proc. Natl. Acad. Sci. USA *101*, 15949–15954.

Iso, T., Hamamori, Y., and Kedes, L. (2003). Arterioscler. Thromb. Vasc. Biol. *23*, 543–553.

Leong, K.G., Hu, X., Li, L., Noseda, M., Larrivee, B., Hull, C., Hood, L., Wong, F., and Karsan, A. (2002). Mol. Cell. Biol. *22*, 2830–2841.

Liu, Z.J., Shirakawa, T., Li, Y., Soma, A., Oka, M., Dotto, G.P., Fairman, R.M., Velazquez, O.C., and Herlyn, M. (2003). Mol. Cell. Biol. *23*, 14–25.

Mailhos, C., Modlich, U., Lewis, J., Harris, A., Bicknell, R., and Ish-Horowicz, D. (2001). Differentiation *69*, 135–144.

Noseda, M., Chang, L., McLean, G., Grim, J.E., Clurman, B.E., Smith, L.L., and Karsan, A. (2004). Mol. Cell. Biol. *24*, 8813–8822.

Orimo, A., Gupta, P.B., Sgroi, D.C., Arenzana-Seisdedos, F., Delaunay, T., Naeem, R., Carey, V.J., Richardson, A.L., and Weinberg, R.A. (2005). Cell *121*, 335–348.

Patel, N.S., Li, J.L., Generali, D., Cranston, D.W., and Harris, A.L. (2005). Cancer Res. *65*, in press.

Sparmann, A., and Bar-Sagi, D. (2004). Cancer Cell *6*, 447–458.

Uyttendaele, H., Ho, J., Rossant, J., and Kitajewski, J. (2001). Proc. Natl. Acad. Sci. USA *98*, 5643–5648.

Zeng, Q., Li, S., Chepeha, D.B., Giordano, T.J., Li, J., Zhang, H., Polverini, P.J., Nor, J., Kitajewski, J., and Wang, C.-Y. (2005). Cancer Cell *8*, this issue.

DOI: 10.1016/j.ccr.2005.06.013

Gankyrin: An intriguing name for a novel regulator of p53 and RB

The RB and p53 tumor suppressors lie at the heart of cancer biology, and inactivation of both pathways is seemingly essential for tumor development. Previous studies identified gankyrin as a component of the 26S proteasome that is consistently overexpressed in liver cancer and promotes cell transformation by binding RB. In the current issue of *Cancer Cell*, Fujita and colleagues (Higashitsuji et al., 2005) show that gankyrin also binds MDM2 and facilitates its destruction of p53. These important findings implicate gankyrin as a dual-purpose negative regulator of RB and p53, thereby identifying gankyrin as a rational cancer therapeutic target.

Persistent proliferation and enhanced cell survival are hallmarks of aggressive tumor cells. Two key proteins that suppress abnormal cell proliferation are the RB and p53 tumor suppressors. RB acts as a brake to block cell cycle progression through its ability to repress E2F, a transcription factor that activates genes essential for the S phase of the cell cycle (for review, see Sherr, 2004). p53 also blocks cell proliferation in part by inducing $p21^{CIP1}$, a protein that binds and inhibits the cyclin/CDK complexes, which are required for progression through the cell cycle. Additionally, p53 induces cell death by activating the expression of genes involved in apoptosis (for review, see Vogelstein et al., 2000). Therefore, not surprisingly, most cancer cells have inactivated both of these factors either directly or indirectly to sever their signaling pathways.

Recently, Fujita and colleagues (Higashitsuji et al., 2000) identified gankyrin as a gene that was consistently overexpressed in human liver cancers. Gankyrin is highly conserved throughout evolution (~40% identity to yeast Nas6P) (Hori et al. 1998) and is localized on human chromosome Xq22.3 in a region where DNA gains are frequently detected in kidney and colon carcinomas. Whether gankyrin expression is deregulated in cancers other than liver tumors is not yet known. Gankyrin contains two special domains consisting of ankyrin repeats and the RB-recognition motif LxCxE (^{178}LACDE^{182}), and derives its name from these features. "Gann" is the Japanese word for cancer, and ankyrin is the functional domain involved in protein-protein interactions.

Gankyrin was initially purified and characterized by Tanaka and coworkers (Hori et al., 1998) as the p28 component of the regulatory subunit of the 26S proteasome, which is an ATP-dependent protease responsible for the degradation of proteins. The observation that gankyrin binds RB, but not p107 or p130 (RB related proteins), in vitro and in vivo when ectopically expressed provided an initial glimpse into the role of gankyrin in tumorigenesis (Higashitsuji et al., 2000). Consistent with these findings, enforced expression of gankyrin in immortalized mouse fibroblasts and human tumor cells conferred growth in soft agar, and this transformation phenotype was dependent on the ability of gankyrin to bind RB (e.g., the LxCxE point mutant E182A is inactive). Interestingly, a splice variant of gankyrin is produced that lacks the LACDE motif, which should render it inactive in targeting RB directly. However, the physiologic role of this variant and how gankyrin gene expression is regulated in general has not yet been explored. Gankyrin facilitates the phosphorylation and degradation of RB (Figure 1), suggesting that increased expression of gankyrin promotes tumorigenicity by targeting RB to the proteasome. Yet gankyrin disrupts RB function by other means as well. Gankyrin also binds cyclin-dependent kinase 4 (CDK4) resulting in a gankyrin-CDK4-Cyclin D ternary complex (Li and Tsai, 2002). In so doing, gankyrin competes with INK4A, an inhibitor of cyclin kinases, for binding to CDK4. Based upon these findings, gankyrin appears to indirectly activate CDK4, resulting in the hyperphosphorylation of RB and concomitant deregulation of E2F1-mediated transcription and cell cycle progression (Figure 1). Taken together, these studies suggest that gankyrin deactivates the RB tumor suppressor pathway at multiple levels, including by direct binding to RB and by ensuring its inactivation through the maintenance of CDK4 kinase activity.

In the present study in *Cancer Cell* by Fujita and colleagues (Higashitsuji et al., 2005), gankyrin is now shown to bind to MDM2, an E3 ubiquitin ligase that negatively regulates p53. Compelling data are provided showing that this interaction occurs naturally between endoge-

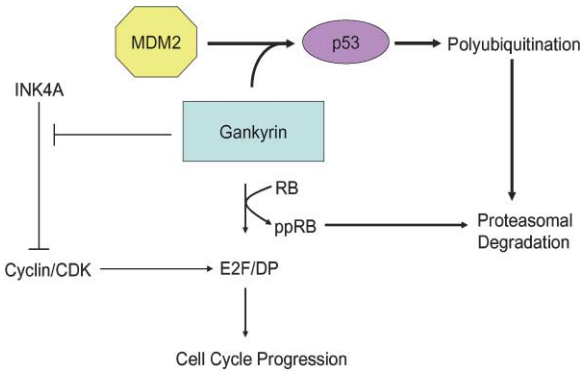

Figure 1. The gankyrin regulatory circuit

Gankyrin interacts with MDM2 and facilitates the ubiquitination and targeting of p53 to the proteasome for degradation. Gankyrin also binds RB to promote RB phosphorylation and degradation. In parallel, gankyrin binds CDK4, which precludes INK4A binding to the cyclin kinase, resulting in enhanced RB phosphorylation and stimulation of E2F transcriptional activity, thus causing cell cycle progression. Gankyrin therefore functions as a dual-negative regulator of the two most prominent tumor suppressor pathways, RB and p53.

nous gankyrin and MDM2 and that gankyrin enhances the ability of MDM2 to ubiquitinate p53. Consequently, gankyrin recruits the MDM2 and p53 complex to the proteasome and fosters the turnover of p53 in an MDM2-dependent manner. Moreover, downregulation of gankyrin expression by RNAi increased p53 protein levels and activity, and promoted apoptosis. These findings indicate that gankyrin functions as a negative regulator of p53 by modulating MDM2 activity. Most importantly, this study provides a plausible mechanism for targeting p53 to the proteasome by MDM2 through its association with gankyrin, an integral component of the 26S proteasome. This study thus expands the role of gankyrin in tumorigenesis by disruption of both the RB and p53 tumor suppressor pathways.

Emerging evidence demonstrates that discrete changes in *MDM2* expression can play a critical role in maintaining normal p53 function and cell physiology. Diminished p53 activity allows increased proliferation and inhibition of apoptosis, providing advantageous signals for tumor cell survival. Too much p53, on the other hand, stifles cell proliferation and leads to apoptosis. Mouse models support these observations. Mice overexpressing *Mdm2* are tumor-prone, while those lacking *Mdm2* die early in embryogenesis due to increased p53 activity (Lozano and Zambetti, 2005). A hypomorphic allele of *Mdm2* further exemplifies the importance of Mdm2 levels in regulating cell proliferation and survival. Mice that express about one-third of the normal Mdm2 levels are small and anemic, with fewer hematopoetic cells (Mendrysa et al., 2003). This phenotype disappears in the absence of *p53*. In humans, *MDM2* expression is modulated by a single nucleotide polymorphism (SNP309) within the promoter region (Bond et al., 2004). This polymorphism results in increased MDM2 levels and thus decreased p53 activity. Individuals who have inherited a defective *p53* allele and the SNP309 develop cancer with significantly shorter latencies as compared to mutant p53 carriers without this polymorphism. Thus, any modulation of MDM2 activity is likely to have consequences on cell survival and tumorigenesis.

As such, the observation presented in the current study, that gankyrin facilitates MDM2 E3 ligase activity, has important implications in tumorigenesis. Decreased activity of gankyrin should ultimately lead to increased p53 levels and decreased risk of cancer. By contrast, an increase in the activity of gankyrin should facilitate MDM2 inactivation of p53 function and lead to a tumor-prone phenotype (Figure 1). Additionally, the ultimate effect on tumorigenesis may be amplified, since gankyrin can also inhibit the activity of the RB tumor suppressor. In this sense, gankyrin imitates the function of transforming proteins such as the Large T antigen from SV40 virus, which binds and inactivates both p53 and RB (Pipas and Levine, 2001).

While these biochemical data provide novel insight into the mechanism by which gankyrin may promote tumorigenesis, many questions remain to be addressed. The recognition that gankyrin binds both Rb and p53 suggests that it may be overexpressed in many tumor types. To date, however, only hepatocellular carcinomas have been examined. Additionally, how does such a small protein (gankyrin is 25 KDa in size) bind RB, Cdk4, MDM2, and the proteasome? Does it do so collectively or independently? Other components of the proteasome may potentially aid in attracting these proteins and shuttling them down the degradation pathway. The intriguing findings presented in the current study (Higashitsuji et al., 2005) implicate gankyrin as a contributory factor in tumor development and highlight this factor as a potentially important therapeutic target. Challenging the functional role of gankyrin in regulating the p53 and RB pathways in a knockout mouse model should yield invaluable insight into its role as a potential modifier of tumorigenesis.

Guillermina Lozano[1] and Gerard P. Zambetti[2,*]

[1]The University of Texas M.D. Anderson Cancer Center
Department of Molecular Genetics
Section of Cancer Genetics
1515 Holcombe Boulevard
Houston, Texas 77030
[2]St. Jude Children's Research Hospital
Department of Biochemistry
332 North Lauderdale
Memphis, Tennessee 38105
*E-mail: gerard.zambetti@stjude.org

Selected reading

Bond, G.L., Hu, W., Bond, E.E., Robins, H., Lutzker, S.G., Arva, N.C., Bargonetti, J., Bartel, F., Taubert, H., Wuerl, P., et al. (2004). Cell *119*, 591–602.

Higashitsuji, H., Itoh, K., Nagao, T., Dawson, S., Nonoguchi, K., Kido, T., Mayer, R.J., Arii, S., and Fujita, J. (2000). Nat. Med. *6*, 96–99.

Higashitsuji, H., Higashitsuji, H., Itoh, K., Sakurai, T., Nagao, T., Sumitomo, H., Masuda, T., Dawson, S., Shimada, Y., Mayer, R.J., and Fujita, J. (2005). Cancer Cell, this issue.

Hori, T., Kato, S., Saeki, M., DeMartino, G.N., Slaughter, C.A., Takeuchi, J., Toh-e, A., and Tanaka, K. (1998). Gene *216*, 113–122.

Li, J., and Tsai, M.-D. (2002). Biochemistry *41*, 3977–3983.

Lozano, G., and Zambetti, G.P. (2005). J. Pathol. *205*, 206–220.

Mendrysa, S.M., McElwee, M.K., Michalowski, J., O'Leary, K.A., Young, K.M., and Perry, M.E. (2003). Mol. Cell. Biol. *23*, 462–472.

Pipas, J.M., and Levine, A.J. (2001). Semin. Cancer Biol. *11*, 23–30.

Sherr, C.J. (2004). Cell *116*, 235–246.

Vogelstein, B., Lane, D., and Levine, A.J. (2000). Nature *408*, 307–310.

DOI: 10.1016/j.ccr.2005.06.014