Unique and Overlapping Functions of GSK-3 Isoforms in Cell Differentiation and Cardiovascular Development*

Published, JBC Papers in Press, December 8, 2008, DOI 10.1074/jbc.R800077200

Thomas Force and James R. Woodgett

From the *Center for Translational Medicine, Cardiology Division, and Program in Cell and Developmental Biology, Thomas Jefferson University, Philadelphia, Pennsylvania 19107 and the **Department of Medical Biophysics, University of Toronto, and Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Ontario MSG 1X5, Canada

Intensive study over the past 30 years has helped define the role of the GSK-3 (glycogen synthase kinase-3) family in a variety of physiological and pathophysiological processes. However, the majority of these studies have relied upon overexpression approaches or nonselective small molecule inhibitors. Herein, we examine recent data derived from studies in gene-targeted embryonic stem cells and knock-out mice in an attempt to define the role these protein kinases play in critical decisions made by stem/progenitor cells and by early lineage-committed cardiomyocytes during development.

The GSK-3 family of serine/threonine kinases was first identified as a negative regulator of glycogen synthase, the rate-limiting enzyme in glycogen synthesis (1, 2). The family consists of two isoforms, α and β, which are 98% identical within their kinase domains but differ substantially in their N- and C-terminal sequences. Unlike most protein kinases, GSK-3 is typically active in unstimulated cells and is inhibited in response to a variety of inputs, including growth factors. Because GSK-3-mediated phosphorylation of substrates usually leads to inhibition of those substrates, the end result of growth factor-mediated inhibition of GSK-3 is typically functional activation of its downstream substrates.

Traditionally, most attention has focused on the β isoform of GSK-3. However, this bias appears to have arisen from two reports suggesting that mammalian GSK-3β was more effective than GSK-3α in rescuing Wnt/wingless pathway defects due to the Zeste-white 3 (GSK-3) mutation in Drosophila (3, 4). However, in these studies, the level of expression of GSK-3α and GSK-3β was not equalized. Indeed, as we will discuss below, GSK-3α and GSK-3β appear to be entirely redundant in terms of regulating Wnt/β-catenin signaling.

Based on studies in isoform-specific knock-outs, both isoforms appear to play key (but not entirely overlapping) roles in metabolism. GSK-3α KO mice demonstrate reduced fat mass with enhanced glucose tolerance and insulin sensitivity due, at least in part, to enhanced glycogen storage in the liver (5). Mice lacking the GSK-3β gene die late in development (6, 7), but tissue-specific knock-outs of GSK-3β have revealed metabolic phenotypes in muscle (but not liver), including enhanced insulin-stimulated glycogen synthase activation and glycogen deposition (8). Moreover, inactivation of GSK-3β in just the β cells of the pancreatic islets is sufficient to rescue insulin resistance and largely prevent the manifestations of diabetes in two different models of the disease in mice: haploinsufficiency of the insulin receptor and deletion of IRS2 (9). The pancreatic β-cell knock-outs also demonstrate preservation of β-islet cells due to enhanced proliferation and reduced apoptosis of these critical insulin-secreting cells (9). Although we will not be focusing on metabolism in this review, as we go forward to discuss ES cell and cardiomyocyte phenotypes, it is important to bear in mind that some part of the phenotypes observed may be driven by alterations in metabolic status in ES cells, cardiomyocytes, or other tissues.

Studies over the past few years have also implicated GSK-3 as a critical regulator of ES cell differentiation in vitro (10–14). However, these studies typically have utilized the small molecule inhibitor BIO, which is not entirely selective for GSK-3 and cannot differentiate isoform-specific effects. More recently, three studies have employed ES cells deleted for GSK-3α, GSK-3β, or both, and findings from these studies clearly support a role for the GSK-3 isoforms in ES cell differentiation. Indeed, the embryonic lethal phenotype seen with deletion of GSK-3β is due to unrestrained proliferation of early committed cardiomyocyte precursors or cardiomyoblasts, which, unlike more fully differentiated cells, retain the capacity to proliferate. Thus, the hyperproliferation is due, in part, to the failure of these cells to adequately differentiate. Herein, we will examine how findings in ES cells in vitro inform us as to mechanisms underlying the findings in the developing heart in vivo.

GSK-3β Promotes ES Cell Differentiation into a Cardiomyocyte Lineage via Wnt/β-Catenin-independent Mechanisms

The concept that GSK-3 isoforms might drive stem cell differentiation was first suggested by Sato et al. (12), who demonstrated that BIO maintained pluripotency of both human and mouse ES cells, leading the authors to propose a possible use for GSK-3 inhibition in regenerative medicine. Of note, the effects

---

* This work was supported, in whole or in part, by National Institutes of Health Grants HL61688 and HL091799 from NHLBI (to T. F.). This work was also supported by Canadian Institutes of Health Research Grants MOP 12858 and 74711 (to J. R. W.). This minireview will be reprinted in the 2009 Mini-review Compendium, which will be available in January, 2010.

1 To whom correspondence should be addressed. E-mail: thomas.force@jefferson.edu.

2 The abbreviations used are: KO, knock-out; ES, embryonic stem; BIO, 6-bromouridine-3′-oxime; LIF, leukemia inhibitory factor; BMP, bone morphogenetic protein; ERK, extracellular signal-regulated kinase; EBs, embryoid bodies; DKO, double knock-out; WT, wild-type; PI3K, phosphatidylinositol 3-kinase; PKB, protein kinase B.
MINIREVIEW: GSK-3 Regulates Stem/Progenitor Cell Functions

of BIO and other GSK-3 inhibitors on ES cells have generally been believed to be mediated by activation of the canonical Wnt/β-catenin pathway, rather than via effects on the activity of other GSK-3 targets. However, based on our studies in ES cells derived from embryos deleted for GSK-3α, GSK-3β, or both and on studies in the developing heart, it is clear that Wnt/β-catenin-independent effects are critically important as well (see below) (7, 15).

Studies on the factors regulating ES cell self-renewal and pluripotency have typically employed empiric formulations of growth media. For example, mouse ES cell self-renewal is sustained by culturing cells in medium containing LIF (a cytokine of the interleukin-6 family that acts via JAK/STAT3 and probably c-Myc) and BMP, acting via Smad proteins and Id (inhibitors of differentiation) proteins (Fig. 1) (Ref. 10 and reviewed in Refs. 13 and 16). Recently, the inductive signal in mouse ES cells leading to lineage commitment that is inhibited by LIF and BMP has been reported to be triggered by FGF4/fibroblast growth factor receptor-mediated activation of the ERK pathway (17, 18).

Not surprisingly, there has been intense interest in defining the components of the so-called “ground state” of ES cell self-renewal, including the signaling pathways that can maintain self-renewal in the absence of exogenous factors such as LIF and BMP (11, 13, 14). Ying et al. (14) recently employed small molecule ATP-competitive kinase inhibitors to define this ground state in mouse ES cells. They utilized inhibitors of the fibroblast growth factor receptor (SU5402), ERKs (PD184352), and a more selective inhibitor of GSK-3 than BIO (CHIR99021) to demonstrate maintenance of self-renewal and pluripotency in the absence of exogenous factors. The contribution of inhibition of GSK-3 activity to maintaining self-renewal was felt to be important when ERK signaling was suppressed by signals transduced by LIF or BMP or by the small molecule inhibitors. Thus, Ying et al. (14) proposed that the primary consequence of reduced GSK-3 activity is to maintain ES cell viability and grow capacity in the setting of persistent inhibition of the cytoprotective ERK pathway by SU5402 and PD184352 (or LIF/BMP) by what they termed “global modulation of the ES cell metabolomic and biosynthetic capacity” (Fig. 1).

Upon examining pluripotent stem cells derived from blastocysts, Chou et al. (11) have challenged the concept of a common ground state. Rather, they proposed that both developmental and environmental (i.e. growth factors) inputs remain central to pluripotency. However, inhibition of GSK-3 (via BIO) is still required.

Because these studies have predominantly employed pharmacologic inhibitors of GSK-3, isoform-specific effects, if any, could not be discerned. To address this limitation, we examined the role of individual GSK-3 isoforms in regulating differentiation of ES cells, specifically focusing on differentiation of ES cells into the cardiomyocyte lineage, an obvious critical step in heart formation during embryogenesis. We studied EBs derived from ES cells deleted for both alleles of either GSK-3α or GSK-3β or all four GSK-3 alleles (DKO) (7, 15).

DKO EBs were markedly impaired in their ability to differentiate and retained large numbers of cells that were Oct4- and Nanog-positive (markers of pluripotency in mouse ES cells) even in the absence of LIF in the culture medium. In contrast, Oct4 and Nanog levels in WT EBs were an order of magnitude lower than in DKO EBs. Furthermore, teratomas derived from DKO ES cells were highly undifferentiated with a carcinomasous appearance. Neuronal tissue was completely absent. DKO EBs also failed to produce any contracting cardiomyocytes, whereas >80% of WT EBs developed regions of spontaneously contracting cardiomyocytes. This failure of DKO EBs to differentiate into cardiomyocytes was partially rescued by lentivirus-mediated gene transfer of wild-type GSK-3α or GSK-3β but not by kinase-inactive mutants of GSK-3.

The failure of DKO ES cells to differentiate into the cardiomyocyte lineage is consistent with the previously proposed concept of the role of Wnt/β-catenin signaling in cardiomyocyte differentiation, i.e. inhibition of Wnt/β-catenin signaling is necessary for cardiomyocyte specification (19–21). In the DKO, β-catenin levels were markedly increased, as was activation of a reporter construct (TOP-Flash) regulated by β-catenin (15). Indeed, based on the lack of neuronal and cardiomyocyte tissue and on the maintenance of pluripotency markers under differentiation conditions, it seems likely that the dominant effect in the DKO cells is dysregulation of Wnt signaling.

Strikingly, in ES cells engineered to lack GSK-3α or GSK-3β but not both, no stabilization of β-catenin occurred, and induction of β-catenin/Tcf-responsive genes was not observed. There was only a minor increase in β-catenin in ES cells lacking both alleles of GSK-3α and one of GSK-3β (15). These data indicate that the two isoforms are not independently rate-limiting in the Wnt pathway and are entirely redundant. Indeed, the limiting factor in this pathway is likely Axin, which does not
discriminate between binding of the two isoforms and is present at significantly lower concentrations than either. Deletion of all four GSK-3 alleles in either ES cells or mouse embryo fibroblasts results in massive up-regulation of \( \beta \)-catenin, as is found in tumor cells that either lack adenomatous polyposis coli or harbor phosphorylation site mutations in \( \beta \)-catenin (reviewed in Ref. 22). In these situations, resulting phenotypes are likely dominated by the deregulation of \( \beta \)-catenin, although the Hedgehog, Notch, and other pathways are similarly derepressed.

Studies in the GSK-3\(^{\text{3a}}\) KO and GSK-3\(^{\text{3β}}\) KO ES cells provided the first opportunity to date to identify Wnt/\( \beta \)-catenin-independent effects of GSK-3\(^{\text{3}}\) isoforms in the regulation of differentiation of ES cells, including differentiation into the cardiomyocyte lineage. Notably, whereas the percent of GSK-3\(^{\text{3a}}\) KO EBs with regions of actively contracting cardiomyocytes was comparable with that of WT EBs, the percent of GSK-3\(^{\text{3β}}\) KO EBs with contracting regions was markedly reduced (7). These findings indicate that GSK-3\(^{\text{3β}}\) promotes cardiomyocyte differentiation via Wnt/\( \beta \)-catenin-independent mechanisms. These results, which were supported by studies examining expression of markers of “stemness” and of cardiomyocyte differentiation, suggest a critical role for GSK-3\(^{\text{3β}}\), particularly in the later stages of differentiation of cardiomyocytes when they begin to express contractile proteins, natriuretic peptides, and calcium-handling proteins (7).

How might the KO of GSK-3\(^{\text{3β}}\) block differentiation if not via effects on Wnt signaling? Although GSK-3\(^{\text{3β}}\) has a host of targets, one candidate regulator of proliferation versus differentiation is c-Myc, which is up-regulated in response to LIF-mediated induction of STAB3 (10). Phosphorylation of Myc at Thr\(^{358}\) by GSK-3\(^{\text{3β}}\) destabilizes c-Myc, leading to its degradation (23). This phosphorylation event has been reported to promote ES cell differentiation, and a T58A mutation, preventing phosphorylation and degradation, maintained pluripotency, even in the absence of LIF (10, 16). In WT ES cells, PI3K likely mediates inhibition of GSK-3 (and stabilization of c-Myc) via activation of PKB/Akt and inhibitory phosphorylation of Ser\(^{\text{9}}\) (GSK-3\(^{\text{3β}}\)) and Ser\(^{\text{38}}\) (GSK-3\(^{\text{3a}}\)), thereby maintaining self-renewal and pluripotency (10, 24–26). More recently, virus- or plasmid-mediated transduction of c-Myc was reported to be a key component of a “mixture” of genes encoding the transcription factors Oct4, Sox2, and Klf4 that allowed the creation of induced pluripotent stem cells derived from fibroblasts (27, 28). All of these findings are consistent with the concept of c-Myc being a key driver of proliferation of ES cells that, as a result, restricts the ability of the ES cells to differentiate (13).

**GSK-3\(^{\text{3β}}\) Regulates Cardiomyoblast Proliferation and Differentiation in Vivo**

Do findings in KO EBs translate into cardiac developmental abnormalities in vivo? GSK-3\(^{\text{3a}}\) KO mice develop normally with no cardiac abnormalities noted at up to 8 weeks of age. In contrast, Hoeﬄich et al. (6) previously reported that targeted deletion of GSK-3\(^{\text{3β}}\) leads to embryonic lethality in a substantial number of embryos secondary to marked hepatic apoptosis. This is due to the failure to recruit cytoprotective NF-\( \kappa \)B signaling in response to tumor necrosis factor-\( \alpha \). However, this phenotype appears to require exposure of the mothers to pathogens that trigger production of tumor necrosis factor-\( \alpha \), which then leads to the death of the embryos. In the absence of pathogen exposure, the great majority of GSK-3\(^{\text{3β}}\) KO embryos survive to late gestation and then die just before or at birth. Examination of the hearts of these embryos revealed normal valve development, endocardial cushion morphology, and neural crest function, suggesting complete or near-complete compensation by GSK-3\(^{\text{3a}}\) for loss of GSK-3\(^{\text{3β}}\) as regards these critical functions. Strikingly, however, both the left and right ventricles were packed with myocytes, with little or no apparent cavity, a phenotype that can be expected to lead to inadequate output of blood from the heart, resulting in heart failure and death (7).

This phenotype was traced to hyperproliferation of cardiomyoblasts in GSK-3\(^{\text{3β}}\) KO embryos. Thus, the inability of GSK-3\(^{\text{3β}}\) KO cardiomyoblasts to fully differentiate appears to allow continued proliferation, leading to a hypertrophic myopathy. This conclusion is consistent with the finding that GSK-3\(^{\text{3β}}\) KO EBs appear to be markedly larger than WT or GSK-3\(^{\text{3a}}\) EBs and have significantly greater mRNA content.

However, the hyperproliferation does not seem to be due solely to a failure to differentiate because GSK-3\(^{\text{3}}\) also has multiple targets that are regulators of cell cycle progression. Indeed, further analysis implicated dysregulation of at least three of these targets (GATA4, D-type cyclins, and Myc family members) as responsible, at least in part, for the hyperproliferative phenotype (Fig. 2). All of these had previously been implicated in regulating cardiomyocyte proliferation during development because deletion of these was associated with thin-walled hearts, which was believed to be secondary to hypoproliferation (29–31). Furthermore, although debate remains (32), all had also been proposed to be regulated by GSK-3 based on studies in various types of cultured cells (33–35). Our studies support the contention that these are bona fide targets in the heart in vivo. Indeed, it appears that reduced signaling through GSK-3\(^{\text{3β}}\) may connect the several growth factors and their receptors at the cell membrane that are known to drive cardiomyoblast proliferation in the developing heart to the transcription factors and cell cycle regulators that transduce the proliferative signals in the nucleus (Fig. 2).

These studies thus conﬁrmed a central role for GSK-3\(^{\text{3β}}\) in the negative regulation of cardiomyocyte proliferation in vivo and demonstrated that GSK-3\(^{\text{3a}}\) cannot compensate for loss of GSK-3\(^{\text{3β}}\) with regard to this function. That this role of GSK-3\(^{\text{3β}}\) in regulating cell proliferation is not restricted to cardiomyocytes is illustrated by the fact that pharmacologic inhibition of GSK-3 has been reported to enhance repopulation of the bone marrow by hematopoietic stem cells (36), although Wnt signaling was believed to be primarily responsible for this. In contrast and consistent with the studies in KO EBs, we saw no evidence of increased activation of canonical Wnt signaling in the developing heart (6, 7), again suggesting a speciﬁc role of GSK-3\(^{\text{3β}}\) in regulating proliferation that is independent of Wnt/\( \beta \)-catenin signaling. Thus, KO of GSK-3\(^{\text{3β}}\) inhibits differentiation, thereby allowing continued proliferation, but proliferation also appears to be directly driven by dysregulation of cell cycle regulators and transcription factors due to the lack of GSK-3\(^{\text{3β}}\) inhibitory
targets. In summary, GSK-3β is necessary for maintaining control of cardiomyocyte proliferation, with lethal effects if that control is lost.

GSK-3β-null embryos also demonstrate double-outlet right ventricle and ventricular septal defects, both common congenital heart defects in humans. Lee et al. (37) recently reported that morpholino-oligonucleotide-mediated knockdown of GSK-3β in zebrafish produced abnormalities of outflow tract positioning, suggesting that this GSK-3 isoform is key to correct outflow tract patterning in both zebrafish and mice. In contrast, morpholino knockdown of either GSK-3α or GSK-3β led to thin-walled hearts, a striking difference compared with the thick-walled hearts in the GSK-3β KO mouse and no phenotype in the GSK-3α KO mouse. The mechanisms of these differences are not clear.

**Implications for Development of GSK-3-targeted Therapeutics**

GSK-3 has been identified as a putative target for the treatment of a very wide range of disorders, including Alzheimer disease, Parkinson disease, diabetes, ischemic injury, inflammation, cancer, and bipolar disorder (5, 9, 38–40). Although there would be little concern over the short-term use of inhibitors such as would likely occur with acute ischemic injury, long-term use would raise theoretical concerns. For example, if inhibition of GSK-3 leads to enhanced stabilization of β-catenin in cancer cells, this could lead to more aggressive malignancies.

**Future Directions**

Over the past year, studies utilizing mice deleted for GSK-3 isoforms have identified a host of effects of these kinases on a number of basic biological processes in a variety of cells and tissues. The studies in ES cells and pluripotent stem cells strongly support the contention first proposed by Sato et al. (12) that strategies to inhibit GSK-3 could play a significant role in regenerative medicine. Similarly, although it is unclear at this time if GSK-3 regulates the biology of induced pluripotent cells, the critical role of the GSK-3 target c-Myc in these cells suggests a role for the kinases as well (27, 28). Specifically for cardiac regenerative medicine, deletion of GSK-3β is sufficient to sustain the proliferative capacity of cardiomyoblasts during development, but the effects of GSK-3 inhibition in the adult heart remain to be determined. It is theoretically possible that inhibition of GSK-3β would have effects on populations of cardiac-resident stem cells similar to the effects it has on cardiomyoblasts of the developing heart. Cardiac-resident stem cells appear to be in short supply, and this may limit their regenerative potential. It will be important to explore the intriguing possibility of being able to induce proliferation of these cells in situ with GSK-3 inhibition, followed by withdrawal of inhibi-
tion, leading to cardiomyocyte differentiation. Whether such a strategy could be used to partially repopulate injured hearts remains to be seen. It will also be important to determine the effects of GSK-3 inhibition on other stem/progenitor cell types shown or posited to be able to differentiate into cardiomyocytes. Finally, defining isoform-specific roles in these cell types as well as in other cells and tissues will be critical because although current small molecule inhibitors do not distinguish between the isoforms, there are unique regions of each that could potentially be targeted, allowing isoform-selective inhibition.

Acknowledgment—We thank Ronglih Liao for thoughtful review of the manuscript.

REFERENCES

1. Doble, B. W., and Woodgett, J. R. (2003) J. Cell Sci. 116, 1175–1186
2. Frame, S., and Cohen, P. (2001) Biochem. J. 359, 1–16
3. Ruel, L., Bourouis, M., Heitzler, P., Pantesco, V., and Simpson, P. (1993) Nature 362, 557–560
4. Siegfried, E., Chou, T. B., and Perrimon, N. (1992) Cell 71, 1167–1179
5. MacAulay, K., Doble, B. W., Patel, S., Hansotia, T., Sinclair, E. M., Drucker, D. J., Nagy, A., and Woodgett, J. R. (2007) Cell Metab. 6, 329–337
6. Hoeflich, K., Luo, J., Rubie, E. A., Tsao, M. S., Jin, O., and Woodgett, J. R. (2000) Nature 406, 86–90
7. Kerkela, R., Kockeritz, L., MaCauly, K., Zhou, J., Doble, B., Beahm, C., Greytack, S., Woulfe, K., Trivedi, C., Woodgett, J. W., Epstein, J. A., Force, T., and Huggins, G. S. (2008) J. Clin. Investig. 118, 3609–3618
8. Patel, S., Doble, B. W., MacAulay, K., Sinclair, E. M., Drucker, D. J., and Woodgett, J. R. (2008) Mol. Cell. Biol. 28, 6314–6328
9. Tanabe, K., Liu, Z., Patel, S., Doble, B. W., Li, L., Cras-Meneur, C., Martinez, S. C., Welling, C. M., White, M. F., Bernal-Mizrachi, E., Woodgett, J. R., and Permutt, M. A. (2008) PLoS Biol. 6, e37
10. Cartwright, P., McLean, C., Sh peppard, A., Rivett, D., Jones, K., and Dalton, S. (2005) Development (Camb.) 132, 885–896
11. Chou, Y. F., Chen, H. H., Eipe, M., Yahachi, A., Chenoweth, J. G., Tesar, P., Lu, J., McKay, R. D., and Geijsen, N. (2008) Cell 135, 449–461
12. Sato, N., Meijer, L., Skalsounis, L., Greengard, P., and Brivanlou, A. H. (2004) Nat. Med. 10, 55–63
13. Silva, J., and Smith, A. (2008) Cell 132, 532–536
14. Ying, Q. L., Fray, J., Nichols, J., Batlle-Morera, L., Doble, B., Woodgett, J. R., Cohen, P., and Smith, A. (2008) Nature 453, 519–523
15. Doble, B. W., Patel, S., Wood, G. A., Kockeritz, L. K., and Woodgett, J. R. (2007) Dev. Cell 12, 957–972
16. Niwa, H. (2007) Development (Camb.) 134, 635–646
17. Kunath, T., Saba-El-Leil, M. K., Almousailleakh, M., Wray, J., Meloche, S., and Smith, A. (2007) Development (Camb.) 134, 2895–2902
18. Stavridis, M. P., Lunn, J. S., Collins, B. J., and Storey, K. G. (2007) Development (Camb.) 134, 2889–2894
19. Eisenberg, L. M., and Eisenberg, C. A. (2006) Dev. Biol. 293, 305–315
20. Naito, A. T., Shiojima, I., Akazawa, H., Hidaka, K., Morisaki, T., Kikuchi, A., and Komuro, I. (2006) Proc. Natl. Acad. Sci. U. S. A. 103, 19812–19817
21. Ueno, S., Weidinger, G., Osugi, T., Kohn, A. D., Golob, J. L., Pabon, L., Reinecke, H., Moon, R. T., and Murry, C. E. (2007) Proc. Natl. Acad. Sci. U. S. A. 104, 9685–9690
22. Polakis, P. (2007) Curr. Opin. Genet. Dev. 17, 45–51
23. Gregory, M. A., Qi, Y., and Hann, S. R. (2003) J. Biol. Chem. 278, 51606–51612
24. Watanabe, S., Umehara, H., Murayama, K., Okabe, M., Kimura, T., and Nakano, T. (2006) Oncogene 25, 2697–2707
25. Paling, N. R., Wheeldon, H., Bone, H. K., and Welham, M. J. (2004) J. Biol. Chem. 279, 48063–48070
26. Storm, M. P., Bone, H. K., Beck, C. G., Bourillot, P. Y., Schreiber, V., Damiano, T., Nelson, A., Savatier, P., and Welham, M. J. (2007) J. Biol. Chem. 282, 6265–6273
27. Okita, K., Nakagawa, M., Hyenjong, H., Ichisaka, T., and Yamanaka, S. (2008) Science 322, 949–953
28. Stadtfeld, M., Nagaya, M., Uitkal, J., Weir, G., and Hochedlinger, K. (2008) Science 322, 945–949
29. Koizumi, H., Trowbridge, J. J., Shi, J., Shigematsu, H., Zagozdzon, A., Sicsinska, E., Geng, Y., Yu, Q., Bhattacharya, S., Bronson, R. T., Akashi, K., and Sicsinska, P. (2004) Cell 118, 477–491
30. Moens, C. B., Stanton, B. R., Parada, L. F., and Rossant, J. (1993) Development (Camb.) 119, 485–499
31. Zeisberg, E. M., Ma, Q., Jurasek, A. L., Moses, K., Schwartz, R. J., Izumo, S., and Pu, W. T. (2005) J. Clin. Investig. 115, 1522–1531
32. Yang, K., Guo, Y., Stacey, W. C., Harwalkar, J., Freethold, J., Hitomi, M., and Stacey, D. W. (2006) BMC Cell Biol. 7, 33
33. Diehl, J. A., Cheng, M., Roussel, M. F., and Sherr, C. J. (1998) Genes Dev. 12, 3499–3511
34. Xiao, Y., Yang, K., Harwalkar, J., Nye, J. M., Mason, D. R., Garrett, M. D., Hitomi, M., and Stacey, D. W. (2006) BMC Cell Biol. 7, 33
35. Sears, R., Nuckolls, F., Eura, T., Taya, Y., Tamai, K., and Nejins, J. R. (2000) Genes Dev. 14, 2502–2514
36. Trowbridge, J. J., Xenocostas, A., Moon, R. T., and Bhatia, M. (2006) Nat. Med. 12, 89–98
37. Lee, H. C., Tsai, J. N., Liao, P. Y., Tsai, W. Y., Lin, K. Y., Chuang, C. C., Sun, C. K., Chang, W. C., and Tsai, H. J. (2007) J. Biol. Chem. 282, 2895–2902
38. Aghdam, S. Y., and Barger, S. W. (2007) Proc. Natl. Acad. Sci. U. S. A. 104, 51606–51612
39. Rowe, M. K., Wiest, C., and Chuang, D. M. (2007) J. Biol. Chem. 282, 6314–6328
40. 25. Paling, N. R., Wheeldon, H., Bone, H. K., and Welham, M. J. (2004) J. Biol. Chem. 279, 48063–48070
41. Liu, S., Fang, X., Hall, H., Yu, S., Smith, D., Lu, Z., Fang, D., Liu, J., Stephens, L. C., Woodgett, J. R., and Mills, G. B. (2008) Proc. Natl. Acad. Sci. U. S. A. 105, 5248–5253
42. Smith, T. K., and Bader, D. M. (2007) Semin. Cell Dev. Biol. 18, 84–89

MINIREVIEW: GSK-3 Regulates Stem/Progenitor Cell Functions