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*J Immunol* 2011; 187:5587-5595; Prepublished online 28 October 2011;
doi: 10.4049/jimmunol.1101257
http://www.jimmunol.org/content/187/11/5587

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Glycogen Synthase Kinase-3β Is a Prosurvival Signal for the Maintenance of Human Mast Cell Homeostasis

Madeleine Rådinger,1 Daniel Smrž, Dean D. Metcalfe, and Alasdair M. Gilfillan

Homeostasis of mature tissue-resident mast cells is dependent on the relative activation of pro- and antiapoptotic regulators. In this study, we investigated the role of glycogen synthase kinase 3β (GSK3β) in the survival of neoplastic and nonneoplastic human mast cells. GSK3β was observed to be phosphorylated at the Y216 activating residue under resting conditions in both the neoplastic HMC1.2 cell line and in peripheral blood-derived primary human mast cells (HuMCs), suggesting constitutive activation of GSK3β in these cells. Lentiviral-transduced short hairpin RNA knockdown of GSK3β in both the HMC1.2 cells and HuMCs resulted in a significant reduction in cell survival as determined with the MTT assay. The decrease in stem cell factor (SCF)-mediated survival in the GSK3β knockdown HuMCs was reflected by enhancement of SCF withdrawal-induced apoptosis, as determined by Annexin V staining and caspase cleavage, and this was associated with a pronounced reduction in SCF-mediated phosphorylation of Src homology 2 domain-containing phosphatase 2 and ERK1/2 and reduced expression of the antiapoptotic proteins Bcl-xl and Bcl-2. These data show that GSK3β is an essential antiapoptotic factor in both neoplastic and nontransformed primary human mast cells through the regulation of SCF-mediated Src homology 2 domain-containing phosphatase 2 and ERK activation. Our data suggest that targeting of GSK3β with small m.w. inhibitors such as CHIR 99021 may thus provide a mechanism for limiting mast cell survival and subsequently decreasing the intensity of the allergic inflammatory response. The Journal of Immunology, 2011, 187: 5587–5595.

C hronic allergic inflammation is characterized by increased mast cell infiltration and population in the affected tissues. Mast cell burden in these tissues is dependent not only on migration of mast cells/mast cell precursors into these sites but also on prosurvival and antiapoptotic signaling pathways. Processes, including the migration and survival of tissue mast cells, are tightly regulated by stem cell factor (SCF), the ligand for the growth factor receptor KIT (1–3). KIT, which is a member of the growth factor receptors with inherent tyrosine kinase family activity (4, 5), undergoes dimerization and autophosphorylation following SCF-induced ligation. Constitutive activation of KIT activity, through a point mutation (D816V) in the KIT catalytic domain is considered a hallmark of the myeloproliferative disorder mastocytosis (6–8). This disease is characterized by dysregulated growth of mast cells and elevated mast cell numbers in associated skin lesions and tissues such as bone marrow (7). In addition to the documentation of more mast cells harboring the D816V mutation within tissues of mastocytosis patients, the rapidly dividing HMC1.2 human mast cell line also expresses this mutation (9).

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Received for publication May 2, 2011. Accepted for publication September 27, 2011.

This work was supported by the Division of Intramural Research of the National Institute of Allergy and Infectious Diseases within the National Institutes of Health. M.R. was supported in part by the VBG-GROUP Centre for Asthma and Allergy Research, Herman Krefting Foundation against Asthma and Allergy.

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Abbreviations used in this article: GS, glycogen synthase; GSK3β, glycogen synthase kinase 3β; HuMC, human mast cell; PI, propidium iodide; PLCγ, phospholipase Cγ; SCF, stem cell factor; shCont, scrambled short hairpin RNA; shGSK3β, short hairpin RNA for glycogen synthase kinase 3β; SHP2, Src homology 2 domain-containing phosphatase 2; shRNA, short hairpin RNA.

www.jimmunol.org/cgi/doi/10.4049/jimmunol.1101257
mast cell survival and apoptotic pathways in neoplastic mast cells and potentially mast cells participating in the allergic inflammatory response.

Materials and Methods

Mast cell culture

HuMCs were developed from CD34+ peripheral blood progenitor cells in StemPro-34 culture medium containing StemPro supplement (Invitrogen Life Technologies), t-glutamine (2 mM), penicillin (100 U/ml), streptomycin (100 µg/ml), recombinant human IL-3 (30 ng/ml, first week only), recombinant human IL-6 (100 ng/ml), and recombinant human SCF (100 ng/ml) (PeproTech, Rocky Hill, NJ). Experiments were conducted 7–9 wk after the initiation of HuMC cultures. The CD34+ cells were obtained from normal volunteers under a protocol (98-L-0027; principal investigator, Dr. A. Kirshenbaum) approved by the National Institute of Allergy and Infectious Diseases Institutional Review Board and with appropriate informed consents. The growth factor-independent human mast cell line HMC.1.2 was cultured in IMDM medium supplemented with FBS (10%), t-glutamine (2 mM), penicillin (100 U/ml), and streptomycin (100 µg/ml).

Lentivirus shRNA transfection of cell lines and transduction of HuMCs

The following GSK3β-targeted shRNA were purchased from Sigma-Aldrich (St. Louis, MO): 5′-CCGGGCACCTAACTGATCCACACTTTT-3′ (TRCN0000010552) (construct A) and 5′-CCGGCACACTAAGTGGATGGAATCTCGAGATT- TCCACATCTGATTGTTTCTT-3′ (TRCN0000040002) (construct B); and 5′-CCGGCACAAAGATGAAGAGCACCAACTCGAGTTGGTTG- CTCCTCATCTTGTGTTTTT-3′ (SHC002) (control nontarget vector). Packaging vector (MissionLentiviral packaging mix; Sigma-Aldrich), pLKO1 transfer vectors with GSK3β shRNA (Sigma-Aldrich), or scrambled control shRNA were cotransfected into 293T packaging cells (vector). Packaging vector (MissionLentiviral packaging mix; Sigma-Aldrich), pLKO1 transfer vectors with GSK3β shRNA (Sigma-Aldrich), or scrambled control shRNA were cotransfected into 293T packaging cells with pBabePuro transfection reagent (Roche, Indianapolis, IN) as described (15). The transfected 293T cells were grown in DMEM containing FBS (10%), t-glutamine (4 mM), penicillin (100 U/ml), and streptomycin (100 µg/ml). Following 16–19 h of transfection, medium was removed and replaced with fresh DMEM. After 62–65 h of transfection, virus was collected by centrifugation, and the virus pellet was resuspended in 3 ml prewarmed StemPro medium (Invitrogen Life Technologies). Transduction of HuMCs was conducted by transferring the 3 ml resuspended virus to a T75 culture flask containing 3 to 4×10⁶ HuMCs in 15 ml supplemented StemPro cell culture medium (Invitrogen Life Technologies) or 2 to 3×10⁶ HMC.1.2 cells in 10 ml IMDM medium. Two days postinfection, the medium was changed to virus-free medium, and antibiotic selection was initiated (0.2 µg/ml puromycin [Sigma-Aldrich] for HuMCs and 2 µg/ml puromycin for HMC.1.2 cells). Experiments were conducted on days 7 to 8 posttransduction. We have previously demonstrated that this construct does not affect the expression of other signaling proteins including phospholipase Cγ (PLCγ1), Lyn, and NF-xB in HuMCs (15).

Cell activation

HuMCs were starved in cytokine-free media overnight following rinsing with HEPES buffer containing 0.04% BSA and activated by addition of SCF (30 ng/ml) for the times noted in the figure legends. Where indicated, cells were pretreated with the Src family tyrosine kinase inhibitor PP2 (30 nm) for 20 min prior to the activation with HEPES buffer containing 0.04% BSA and activated by addition of SCF (30 ng/ml) for the times noted in the figure legends. Where indicated, cells were pretreated with the Src family tyrosine kinase inhibitor PP2 (3 µM), the PLCγ inhibitor U73122 (1 µM; AH Diagnostics, Aarhus, Denmark) for 20 min prior to the activation with SCF. The PLCγ inhibitor wortmannin (100 nM; Calbiochem) 20 min prior to the activation with SCF. Having successfully knocked down GSK3β in the HMC.1.2 cells, we next determined the consequences of GSK3β knock-
down on the proliferation and/or survival of HMC1.2 cells. As shown in Fig. 1B, GSK3β knockdown significantly decreased the number of viable HMC1.2 cells as assessed by trypan blue dye exclusion 9 d posttransduction. In addition, when cell survival was determined by the MTT assay in which the starting cell number was 1 × 10^5 cells/well, proliferation was assessed by BrdU incorporation (Fig. 1C). To determine whether this reduction may, in part, reflect a decrease in proliferation rate, HMC1.2 cells, transduced with shRNA targeting GSK3β or with scrambled control shRNA, were cultured overnight in the absence of FBS, resuspended in media containing FBS for 24 h, and cell proliferation monitored by BrdU incorporation. As shown in Fig. 1D, BrdU incorporation was significantly reduced in the GSK3β knockdown in HMC1.2 cells compared with the scrambled control shRNA-treated cells. Taken together, these data suggest that GSK3β required for HMC1.2 cell survival, but this, in part, may reflect a requirement for GSK3β in cell proliferation. To further investigate this potential role for GSK3β in mast cell homeostasis, we next determined the manifestations of GSK3β knockdown in primary human mast cells.

Expression and phosphorylation of GSK3β in primary human mast cells

We first confirmed the ability of GSK3β-targeted shRNA to downregulate GSK3β expression and phosphorylation in SCF-challenged and quiescent HuMCs. Cells, starved of cytokines overnight and treated with control or GSK3β-targeted shRNA, were either unchallenged or challenged with SCF for 2 min and cell lysates assessed for expression and phosphorylation of GSK3β. Despite overnight starvation of SCF, and as was observed in the HMC1.2 cells, GSK3β was found to be constitutively phosphorylated at position Y216 in resting HuMCs and no consistent increase in the phosphorylation of this residue was observed in cells rechallenged with SCF (Fig. 2A). Regardless, the expression of GSK3β and, as a consequence, GSK3β phosphorylated at Y216 was markedly reduced in HuMCs treated with GSK3β-targeted shRNA. In contrast to the HMC1.2 cells, there was minimal constitutive phosphorylation of the S9 residue of GSK3β in resting primary HuMCs. However, this phosphorylation was enhanced in SCF-challenged cells (Fig. 2A). As expected, this phosphorylation was markedly reduced in cells treated with GSK3β-targeted shRNA. Nevertheless, the inability of SCF alone to further enhance the observed constitutive phosphorylation of GSK3β at Y216 again suggests that GSK3β may be constitutively active in the resting state in HuMCs and that this activity cannot be further enhanced through KIT activation. Furthermore, the lack of reduction of the phosphorylation of GS by SCF (Fig. 2B), a response that could be reduced by GSK3β-targeted shRNA (15), suggests that the phosphorylation of GSK3β at S9 in the HuMCs minimally impacted GSK3β activity, at least over the time frame examined.

FIGURE 1. Antiproliferative effect of GSK3β knockdown in HMC1.2 cell line. A, The HMC1.2 cell line was transduced with shContr or shGSK3β. Whole-cell extracts were prepared and immunoblotted with anti-GSK3β, anti–phospho-GSK3α/β (p-Y279/Y216), anti–p-GSK3β (S9), anti-Syk, or anti–KIT Abs. B, Cell viability was assessed by trypan blue dye exclusion. Cell survival was assessed by means of an MTT assay in which the starting cell number was 1 × 10^5 cells/well (C), and proliferation was assessed by BrdU incorporation (D). In all cases, n = 3–5. *p < 0.05, **p < 0.01 for comparison with shContr-transduced HMC1.2.

FIGURE 2. Expression and activity of GSK3β inhibition in primary HuMCs. A, HuMCs, transduced with shContr or shGSK3β, were starved overnight in SCF-depleted media and then stimulated with SCF for 2 min as described in Materials and Methods. Whole-cell extracts were prepared and immunoblotted with anti–p-GSK3β (Y216), anti–p–GSK3β (S9), or anti–GSK3β Abs. The level of Syk demonstrates equivalent protein loading of samples. B, Kinetics of SCF-mediated phosphorylation of GSK3β in HuMCs. Whole-cell extracts were prepared and immunoblotted with anti–p-GSK3α/β (p-Y279/Y216), anti–p–GS (pGS) (S641), or anti–p-GSK3β (S9) Abs. The level of total Syk demonstrates equivalent protein loading of samples. C, HuMCs were preincubated with a Src inhibitor (PP2), a PLCγ inhibitor (U73122), or a PI3K inhibitor (wortmannin [Wortm]) for 20 min prior to stimulation with SCF for 2 min as described in Materials and Methods. Whole-cell extracts were prepared and immunoblotted with anti–p–Akt (S473), anti–p–GSK3β (Y216), anti–p–GSK3β (S9), or anti–p–GS (S641) Abs. The level of Syk demonstrates equivalent protein loading of samples.
We have previously determined that phosphorylation of the downstream substrate for GSK3β, GS, is moderately enhanced in HuMCs following a combination of KIT and FcεRI-mediated activation (15). Hence, we examined the phosphorylation status of p-GSK3β (Y216) and GS (S641) in HuMC over a 10-min time period during which maximal SCF-induced KIT phosphorylation is observed (24). As can be seen from Fig. 2B, there was no consistent increase in phosphorylation of either of these respective GSK3β and GS residues. These data overall are thus consistent with the conclusion that the constitutive phosphorylation and activity of GSK3β observed in the HMC1.2 and HuMC cells is regulated at least in part independently of KIT activity.

To provide further support for this conclusion, we investigated whether inhibitors of known signaling processes downstream of KIT block the constitutive phosphorylation of GS and GSK3β, including PP2, an Src family tyrosine kinase inhibitor also capable of inhibiting KIT phosphorylation (24); U73122, a PLCγ1 inhibitor (25); and wortmannin, a selective PI3K inhibitor (25). As expected, and to serve as a positive control, PP2 inhibited SCF-mediated PI3K activation, as indicated by the attenuation of the phosphorylation of Akt. Wortmannin primarily inhibited Akt phosphorylation, whereas U73122 partially blocked Akt phosphorylation (Fig. 2C). Although all three agents variably inhibited the increase in phosphorylation of GSK3β at S9 and the observed increase in phosphorylation in GS at S641 in these experiments, they had minimal effect on the constitutive phosphorylation of GSK3β at Y216 and GS at S641. These data suggest that although KIT activates PLCγ1 (25), tyrosine kinases, and PI3K, the constitutive activation pathway is independent of these signaling processes. These data further support the conclusion that the constitutive activation of GSK3β observed in mast cells is independent of KIT activation.

**GSK3β is required for SCF-mediated HuMCs survival**

To next determine the outcome of GSK3β knockdown on HuMC survival, two different shRNA constructs were selected based on their relative abilities to decrease GSK3β expression in HuMCs as described (15) and as shown in Fig. 3A. Knockdown of GSK3β with these constructs resulted in a significant reduction in viable cells as determined by trypan blue viability count (68.9 ± 8.6% construct A) and 31.2 ± 10.3% construct B) versus 91.3 ± 2.7% (control construct) remaining viable cells; n = 3, p < 0.01) (Fig. 3B) and with the MTT assay (54.4 ± 10.4% and 25.7 ± 7.1% versus 95.8 ± 3.3% for survival of cell input; n = 3, p < 0.05 and p < 0.001, respectively) (Fig. 3C). Based on these parameters, the decrease in survival of the cells treated with the two different GSK3β constructs thus correlated to the degree of GSK3β knockdown observed in Fig. 3A. Taken together, these data indicate that, although the requirements for GSK3β in the maintenance of neoplastic mast cells may be partly dependent on effects on cell division, at least in mature primary cultured HuMCs, it principally functions as an antiapoptotic signal for the maintenance of mast cell survival.

**GSK3β knockdown increases SCF withdrawal-induced apoptosis in HuMCs**

To provide further evidence to support the role of GSK3β as an antiapoptotic factor, we next determined whether the degree of knockdown of GSK3β similarly correlated to the respective markers of apoptosis and cell death, Annexin V and PI staining. The GSK3β-targeted shRNA-treated cells were grown in SCF-depleted medium for 20 h to increase the sensitivity of the assay. Under these conditions, the shGSK3β-treated HuMCs displayed a significant increase in apoptosis compared with the scrambled control-treated cells (construct A: 39.9 ± 2.7% and construct B: 54.5 ± 3.9% versus 25.6 ± 3.1% apoptotic cells; n = 4 to 5, p < 0.001) (Fig. 4A). Fig. 4B shows representative scatter plots of HuMCs transduced with shContr or shRNA for two different constructs of GSK3β starved in SCF-depleted media for 20 h and stained for Annexin V and PI. Caspase-3, which is activated upon cleavage, plays a dominant role in the extrinsic apoptotic pathway. To further establish the role of GSK3β in the extrinsic apoptotic pathway, we therefore next examined cleaved caspase 3 by Western blotting. As can be seen in Fig. 4C and 4D, the increase in apoptotic cells was associated with an increase in cleaved caspase-3 in the GSK3β knockdown cells compared with control-treated cells. Taken together, these data support a role for GSK3β as a key regulator of an antiapoptotic signaling pathway required for mast cell homeostasis. These data further support the conclusion that the antiapoptotic signals provided by GSK3β act in conjunction with those initiated by SCF, rather than being directly regulated by SCF-mediated signaling.
Downregulation of GSK3β inhibits SHP2 and ERK1/2 but not PI3K activation

As discussed (26, 27), an intact PI3K signaling axis is required for mast cell homeostasis. We therefore next examined whether the similar requirement for GSK3β for the maintenance of mast cell homeostasis could be explained by potential positive-feedback regulation of PI3K activity. We thus examined if the SCF-dependent phosphorylation of Akt was inhibited in the GSK3β knocked-down HuMCs compared with the scrambled control-treated cells. As expected, and by means of a control, both the phosphorylation of Y216 of GSK3β and the phosphorylation of S9 of GSK3β were significantly reduced in the knockdown cells compared with control shRNA-treated cells (Fig. 5A–C). In contrast, there was no significant difference in the phosphorylation of Akt (S473) (Fig. 5A, 5D) following SCF stimulation in the GSK3β knockdown cells compared with control cells. These observations indicate that the decrease in survival of HuMCs following knockdown of GSK3β was not due to positive feedback regulation of PI3K by GSK3β, but requires the regulation of other critical signaling elements required for mast cell homeostasis.

In addition to PI3K, studies conducted in mouse bone marrow-derived mast cells suggest that SHP2 is also required for SCF-mediated mast cell proliferation and survival through the regulation of Rac/JNK (28). Furthermore, SHP2/Ras activation has also been shown to be critical for growth factor-induced ERK activation through an undefined mechanism (29). We thus investigated whether the impact of GSK3β deficiency on HuMC survival may be explained by a downregulation of SHP2 and/or ERK activity. As can be seen in Fig. 6A–C, SCF-mediated activation of both SHP2 and ERK was significantly reduced in the GSK3β knockdown cells compared with the control cells. It is also of note that the constitutive phosphorylation of SHP2 but not ERK tended to also be lower in GSK3β knockdown cells. Taken together, these data indicate that the constitutive activation of GSK3β maintains mast cell homeostasis by providing a permissive signal allowing phosphorylated KIT to recruit SHP2 and ERK.

Knockdown of GSK3β downregulates expression of Bcl-2 family member antiapoptotic proteins

Growth factor-induced apoptosis is regulated by the interplay of prosurvival and proapoptotic members of the Bcl-2 family proteins (30, 31). Studies have demonstrated that hyperactivation of phospho-ERK enhances cell survival by inducing expression of the prosurvival molecules Bcl-2 and Bcl-xL and suppression of the proapoptotic BH3-only protein Bim (32, 33). Similarly, gain-of-function SHP2 mutant-expressing cells have elevated levels of
both Bcl-2 and Bcl-xL and reduced levels of Bim (32), which has previously been shown to be critical for growth factor deprivation-induced mast cell apoptosis (34). As we observed that GSK3β contributed to the regulation of ERK and SHP2 activity, we examined the expression of both antiapoptotic (Mcl-1, Bcl-xL, and Bcl-2) and proapoptotic (Bim, Bid, and Bad) proteins in the SCF-starved and nonstarved HuMCs following treatment with the GSK3β-targeted and control shRNA. As shown in Fig. 7 A, GSK3β knockdown, if anything, increased the expression of Mcl-1, whereas SCF starvation reduced the expression of this anti-apoptotic protein. However, the expression of Bcl-xL and Bcl-2 were reduced by both SCF starvation and GSK3β knockdown, with further reduction observed with the combination of these approaches. In contrast, whereas SCF-enhanced the expression of Bim (Fig. 7B) but partially reduced the expression of Bid and Bad, GSK3β knockdown reduced the expression of all three proteins. Nevertheless, the data as a whole suggested that GSK3β acts as a prosurvival signal for human mast cells by regulating the expression of the antiapoptotic proteins Bim, Bid, and Bad and, to a certain extent, by SCF starvation (Bid and Bad) was somewhat counterintuitive but may be a consequence of caspase cleavage (35, 36).

A small-molecule GSK3β inhibitor decreases survival of the HMC1.2 human leukemia mast cell line

Finally, based on our conclusion that GSK3β was an important mast cell prosurvival signal, we investigated whether a small molecule inhibitor of GSK3β could be employed to reduce HMC1.2 survival. To date, CHIR 99021 is the most selective inhibitor of GSK3β reported (37, 38). As can be seen in Fig. 8, this compound dose-dependently reduced the number of viable HMC1.2 cells after 24, 48, and 72 h in culture, thus providing evidence for
Although it is apparent that SCF can induce the phosphorylation of AKT at S473, which has been shown to be critical for mast cell homeostasis, our observations that the phosphorylation of AKT is unaffected by GSK3β knockdown (Fig. 5) suggests that GSK3β does not regulate cell proliferation/survival through the feedback regulation of PI3K activity. It thus appears more likely that the ability of GSK3β to modulate the phosphorylation of AKT is unaffected by GSK3β knockdown and the indices of apoptosis, Annexin V staining, and caspase cleavage in the SCF-starved HuMCs (Fig. 4).

The requirement for GSK3β in mast cell homeostasis may be dependent on both its regulation of cell division and of anti-apoptotic pathways. Although the BrdU assay conducted in the HMC1.2 cells indicates that the dependency of the survival of these cells on GSK3β could be partly explained by prevention of cell division, the studies conducted on the nonproliferating HuMCs would indicate that GSK3β also plays a major role in the prevention of mast cell apoptosis. This conclusion is supported by the close correlation between GSK3β expression and HuMC survival as determined by trypan blue exclusion and the MTT assay (Fig. 3) and by the close correlation between the degree of GSK3β knockdown and the indices of apoptosis, Annexin V staining, and caspase cleavage in the SCF-starved HuMCs (Fig. 4).

GSK3β has been described to regulate multiple cellular events including cell growth, cell survival, metabolism, gene expression, and apoptosis (21, 41–43). However, in certain cases, including the regulation of growth factor- and mitogen-mediated responses, the described roles appear paradoxical in that GSK3β may both positively and negatively regulate cellular processes through tightly coupled activation and/or inactivation. For example, in a variety of cell types including eosinophils (41), GSK3β has been suggested to support cell survival downstream of the PI3K/Akt pathway. However, in studies conducted in both hematopoietic cells and in neuronal cells, it has been proposed that apoptosis induced by growth factor withdrawal or PI3K inhibition is mediated by GSK3β (44–46). Furthermore, GSK3β inhibition has been suggested to modulate radiation resistance in certain cancers as well as promoting tumor growth through stabilization of B-catenin (16, 47). Thus, as with the regulation of GSK3β activity, GSK3β-regulated responses may also represent a fine balance between negatively regulated and positively regulated signaling responses.

Our previous studies in which we explored the regulation of mast cell chemotaxis and cytokine production by GSK3β (15) also indicate that the role(s) of GSK3β in mast cell function are complex. Our results suggested, for example, that constitutively activated GSK3β must be considered in the context of regulation of signaling events in addition to the potential roles of receptor-mediated upregulation and/or downregulation of GSK3β activity (15). Certainly, in the current study, the constitutive activity of GSK3β we observed, the inability of SCF to enhance this response, and the requirement for ongoing GSK3β activity for cell survival would suggest that GSK3β is also a prerequisite signal, rather than an inducible signal, for mast cell homeostasis.

Although PI3K and PI3K-regulated prosurvival pathways are recognized to be critical for mast cell homeostasis, our observations that the phosphorylation of AKT is unaffected by GSK3β knockdown (Fig. 5) suggests that GSK3β does not regulate cell proliferation/survival through the feedback regulation of PI3K activity. However, based on our previous results (15), it is possible that PI3K may, in part, contribute to the regulation of GSK3β activity. It thus appears more likely that the ability of GSK3β to function as a prosurvival factor is related to its requirement for the ability of SCF to induce SHP2 and ERK phosphorylation (Fig. 6) and potentially JNK phosphorylation (15). In mouse bone marrow-derived mast cells, SHP2, through the regulation of Rac/JNK, has been shown to be required for SCF-mediated mast cell prolifera-
tion and survival (28) and to be critical for growth factor-induced ERK activation (48). SHP2 is a ubiquitously expressed non-receptor protein tyrosine phosphatase that participates in signaling events downstream of receptors for growth factors, cytokines, hormones, and control cell growth, differentiation, migration, and death (48). Thus, activation of SHP2 and its association with Gab1 is critical for sustained ERK activation downstream of several G protein-coupled receptors and cytokines (48). The necessity for GSK3β in regulation of the MAPKs ERK and JNK in the manner described above may be due to priming of regulatory components of the MAPK pathways by GSK3β (15). Consequently, a deficiency in GSK3β would result in an inability of SCF, or indeed other stimuli, to regulate cellular responses via the MAPKs. However, it is also possible that SHP2 acts as a GSK3β substrate, because GSK3β-knockdown cells showed an impaired SCF-mediated activation of SHP2. As both SHP2 and ERK have been demonstrated to regulate the expression of antiapoptotic Bcl-2 family proteins (32, 33), our observation that GSK3β knockdown resulted in the downregulation of these proteins (Fig. 7) provided evidence for the mechanism by which GSK3β may function as a prosurvival signal in human mast cells. Such a mechanism would involve the requirement of SHP2 and ERK for constitutively active GSK3β to promote cell survival through expression of the Bcl-2 family members Bcl-2 and Bcl-xL.

In summary, the data presented in this study provide evidence that GSK3β is a key regulator of mast cell homeostasis in both neoplastic and primary cultured human mast cells through prevention of apoptosis. The data also indicate that the myeloproliferative capacity of the neoplastic HMC1.2 cells at least in part requires GSK3β activity and that a small-molecule inhibitor of GSK3β (CHIR 99021) of GSK3β activity effectively reduces HMC1.2 cell survival (Fig. 8). Thus, targeting GSK3β may provide a mechanism for modulating mast cell survival and apoptotic pathways in myeloproliferative disorders as well as in the allergic inflammatory response.

Acknowledgments

We thank the clinical staff within the Laboratory of Allergic Diseases of the National Institute of Allergy and Infectious Diseases/National Institutes of Health for providing the CD34+ cells for culture of HuMCs.

Disclosures

The authors have no financial conflicts of interest.

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