

A TRAIL receptor-dependent synthetic lethal relationship between *MYC* activation and *GSK3 β* /*FBW7* loss of function

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The *MYC* protooncogene is frequently deregulated in human cancers. Here, by screening a kinase-directed library of small inhibitory RNAs, we identify glycogen synthase kinase 3 β (*GSK3 β*) as a gene whose inactivation potentiates TNF-related apoptosis-inducing ligand death receptor-mediated apoptosis specifically in *MYC*-overexpressing cells. Small inhibitory RNA-induced silencing of *GSK3 β* prevents phosphorylation of *MYC* on T58, thereby inhibiting recognition of *MYC* by the E3 ubiquitin ligase component *FBW7*. Attenuating the *GSK3 β* -*FBW7* axis results in stabilization of *MYC*, up-regulation of surface levels of the TNF-related apoptosis-inducing ligand death receptor 5, and potentiation of death receptor 5-induced apoptosis *in vitro* and *in vivo*. These results identify *GSK3 β* and *FBW7* as potential cancer therapeutic targets and *MYC* as a critical substrate in the *GSK3 β* survival-signaling pathway. The results also demonstrate paradoxically that *MYC*-expressing tumors might be treatable by drug combinations that increase rather than decrease *MYC* oncoprotein function.

FBW7 | *MYC* | TNF-related apoptosis-inducing ligand

The *MYC* protooncogene encodes a basic helix-loop-helix leucine zipper transcription factor that plays a central role in promoting the development of many human cancers (1, 2). In addition to driving cancer cell growth and proliferation, *MYC* is also capable of sensitizing cells to apoptosis (3, 4), an activity that we recently proposed might be exploitable pharmacologically to selectively kill *MYC*-expressing tumors (5). We showed that *MYC* activation results in up-regulation of cell surface levels of death receptor 5 (DR5; also known as TRAIL-R2, TNFRSF10B), an apoptosis-inducing receptor for the cytokine TNF-related apoptosis-inducing ligand (TRAIL) (reviewed in refs. 6 and 7). *MYC*-overexpressing cells can therefore be killed preferentially over isogenic normal cells by agonists of DR5 apoptotic signaling. This *MYC*-induced apoptotic sensitivity may be a primary mechanism underlying TRAIL's unusual ability, unique among the TNF family of death ligands, to induce apoptosis in tumor cells preferentially over normal cells (5, 8–11).

Recombinant human TRAIL and agonistic antibodies against its two death-inducing receptors, DR4 and DR5, are currently undergoing development as cancer therapeutics. However, because many tumors, including *MYC*-expressing tumors, are resistant or only weakly sensitive to their effects (10), it would be desirable to identify agents that potentiate TRAIL-induced apoptosis. Here, to this end, we screened a library of small inhibitory RNAs (siRNAs) directed primarily against the protein kinase superfamily to identify genes whose inactivation potentiates DR5-mediated apoptosis specifically in *MYC*-expressing cells. This screen can be thought of as a sensitized synthetic lethal genetic screen (12, 13) in which the phenotypic output, lethality, is sensitized not only by a genetic alteration, *MYC* activation, but also by an environmental condition, i.e., by the presence of a suboptimal dose of DR5 agonistic antibody. Among the genes identified in this screen was glycogen synthase kinase 3 β (*GSK3 β*), which plays a central role in a wide variety of physiological and pathological processes (14, 15). We show that *GSK3 β* 's ability to phosphorylate *MYC* and target it for recogni-

tion by the E3 ubiquitin ligase component and tumor suppressor *FBW7* (also known as *FBXW7*, *hCDC4*, *AGO*, and *SEL10*) underlies its ability to potentiate apoptosis selectively in *MYC*-expressing cells. These results identify the *GSK3 β* -*FBW7* axis as a potential *MYC*-specific cancer therapeutic target and demonstrate a counterintuitive approach toward cancer therapy, that of potentiating apoptosis by increasing rather than decreasing oncogene function.

Methods

Cell Lines. Genetically defined immortalized HA1E cells were obtained from Robert Weinberg (Whitehead Institute, Cambridge, MA), HCT116 and its *FBW7*^{+/-} and *FBW7*^{-/-} derivatives were obtained from Bert Vogelstein (The Johns Hopkins University, Baltimore), and HT115 cells were obtained from the European Collection of Cell Cultures (Salisbury, U.K.).

siRNA Library Screening. siRNAs were arrayed in 384-well microtiter plates in duplicate at 8 ng per well (8). For each 384-well plate the signal was normalized by dividing each well by the average of 24 wells on the same plate containing siRNAs against luciferase (siGL3) and multiplying by 100 to obtain normalized viability (percent viability).

Viability Assays. Cells (8,000–10,000 per well) were plated in 96-well plates in the appropriate cell culture medium, incubated with LiCl or KCl for 8 h or transfected with siRNAs, and treated with DR5-A 48 h after transfection for an additional 20 h. Cell viability was measured in triplicate by CellTiterGlo (Promega) according to the manufacturer's instructions.

Supporting Information. Further details are available in *Supporting Methods*, which is published as supporting information on the PNAS web site.

Results

An siRNA Screen Identifies *GSK3 β* as a *MYC*-Dependent Potentiator of DR5-Mediated Apoptosis. To identify genes that modulate cell survival in a *MYC*-dependent fashion, we screened an arrayed library of 624 largely kinase-directed siRNAs (8) for their ability to differentially potentiate DR5-mediated apoptosis in an isogenic pair of cell lines differing only in their level of *MYC* expression (Fig. 1*a*). The siRNA library was reverse-transfected into the immortalized but nontransformed kidney epithelial cell line HA1E or into its derivative, HA1E-*MYC*, which ectopically expresses *MYC* from

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Abbreviations: siRNA, small inhibitory RNA; siGL3, siRNA directed against luciferase; DR5, death receptor 5; TRAIL, TNF-related apoptosis-inducing ligand.

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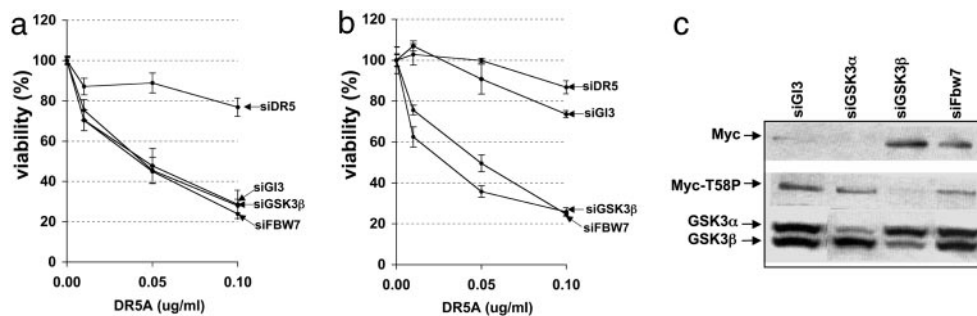


Fig. 3. GSK3 β regulates sensitivity to DR5-mediated apoptosis via phosphorylation of MYC T58. (a and b) Mutation of the GSK3 β MYC T58 phosphorylation site mimics GSK3 β or FBW7 loss of function. Derivatives of HA1E cells expressing retrovirally transduced WT MYC (b) or the T58 phosphorylation site mutant MYCT58A (a) were transfected with the indicated siRNAs and treated with DR5-A and assayed for viability (see Fig. 10 a and b for comparison with higher DR5-A concentrations). (c) In HA1E-MYC cells transfected with siRNAs directed against GSK3 α (siGSK3 α), GSK3 β (siGSK3 β), or FBW7 (siFBW7), MYC protein levels and T58 phosphorylation status were determined by Western blot analysis using MYC or MYCT58-phospho-specific antibodies. By densitometry, siRNAs directed against GSK3 α , GSK3 β , and FBW7 reduced MYC T58 phosphorylation by 1.7-fold, >100-fold, and 1.5-fold, respectively, relative to siG13.

The GSK3 β –FBW7 MYC Degradation Pathway Determines Sensitivity to DR5-A-Induced Apoptosis. Phosphorylation of MYC at T58 by GSK3 β initiates a series of posttranslational modifications that allows it to be recognized and targeted for degradation by FBW7, a tumor-suppressor protein and F-box-containing component of the SCF (Skp–Cullin–F-box) ubiquitin ligase complex (20–22). To determine whether FBW7-dependent degradation of MYC plays a role in the control of cell survival mediated by GSK3 β , we tested whether suppression of FBW7 function would mimic depletion of GSK3 β . siRNAs targeting *FBW7* and *GSK3 β* increased MYC protein levels and enhanced sensitivity to DR5-A to a similar extent in HA1E-MYC cells (Fig. 4 a and b) but had little effect in HA1E cells (Fig. 4 c and d), suggesting that GSK3 β and FBW7 act in a common pathway regulating MYC stability and apoptosis. Consistent with this idea, a functional siRNA against *FBW7*, like those against *GSK3 β* , failed to further sensitize HA1E cells expressing stabilized MYC (HA1E-MYCT58A) (Fig. 3a).

Notably, both *GSK3 β* and *FBW7* siRNAs enhanced MYC protein levels in HA1E-MYC cells to a similar extent as the chemical GSK3 inhibitors (compare Fig. 4b with Fig. 9b). Whereas siRNAs targeting *GSK3 β* prevented MYC T58 phosphorylation, *FBW7* specific siRNAs did not (Fig. 3c), in accordance with observations that FBW7 functions downstream of GSK3 β -dependent MYC T58 phosphorylation (21, 22). Combined with our previous observations that MYC overexpression sensitizes cells to DR5-mediated apoptosis (5), our results suggest that stabilization and accumulation of MYC protein resulting from the inactivation of the GSK3 β /FBW7 axis is responsible for the hypersensitivity of MYC-expressing cells to DR5-A-induced apoptosis.

Mutation of the *FBW7* Tumor Suppressor Enhances Apoptotic Sensitivity in MYC-Expressing Tumor Cells. The experiments presented thus far were performed in derivatives of normal primary cells engineered to overexpress MYC. Importantly, these results could also be recapitulated in human tumor-derived cell lines that endogenously express MYC. Like HA1E-MYC cells, the human colon carcinoma cell line HCT116 could be sensitized to DR5-A by siRNAs against *GSK3 β* and *FBW7*, but not *GSK3 α* (Fig. 5a and Fig. 11a, which is published as supporting information on the PNAS web site). Furthermore, derivatives of HCT116, in which one or both copies of *FBW7* were disrupted by homologous recombination (*FBW7*^{+/-} and *FBW7*^{-/-}, respectively) (Fig. 11b) (23), were hypersensitive to DR5-A when compared with the parental HCT116 line (Fig. 5a). In *FBW7*^{-/-} cells, *GSK3 β* siRNA was unable to further increase apoptotic sensitivity (Fig. 5b and Fig. 12, which is published as supporting information on the PNAS web site), confirming that, in these cells, GSK3 β and FBW7 function in a linear pathway to control the cellular response to DR5 signaling. Interestingly, both

heterozygous and homozygous disruption of *FBW7* strongly enhanced apoptosis by DR5-A (Fig. 5a), consistent with the observation that *FBW7* can act as a haploinsufficient tumor-suppressor gene (24).

Similarly, the colon carcinoma cell line HT115, which carries a naturally occurring heterozygous mutation in *FBW7* was highly sensitive to DR5-A (Fig. 13, which is published as supporting information on the PNAS web site). The mutation present in HT115, C1153T, is among the most frequently occurring *FBW7* mutations found in colon cancer (23), resulting in a mutated arginine residue (R465) critical for substrate recognition (23, 25). Notably, sensitivity to DR5-A could be reversed by stable overexpression of a WT *FBW7* cDNA in HT115 cells (Fig. 13).

In HCT116 and HT115 cells, as in HA1E-MYC cells, the increased sensitivity to DR5-A caused by *FBW7* mutation critically depended on MYC levels, given that knockdown of MYC by siRNA could suppress DR5-A-induced apoptosis in these cell lines (Fig. 5c and Fig. 14, which is published as supporting information on the PNAS web site). Qualitatively similar rescue in the HCT116 series of cell lines was also obtained if MYC function was reduced by stable retroviral expression of a dominant negative MYC allele (26) (Fig. 15, which is published as supporting information on the PNAS web site). In contrast, an siRNA that reduced levels of cyclin E, another substrate of GSK3 (27) and FBW7 (28), had no significant effects on cell viability (Fig. 5c), despite strongly suppressing micronuclei formation (a proxy for chromosomal instability) induced by *FBW7* disruption (Fig. 16, which is published as supporting information on the PNAS web site) as reported in ref. 23. Because MYC siRNAs had no influence on micronuclei formation in *FBW7*-depleted cells (Fig. 16), our data delineate the function of two critical FBW7 targets, MYC and cyclin E: In *FBW7*-depleted cells, the resulting increase in DR5-A sensitivity depends on MYC stabilization, whereas the increase in chromosomal instability depends on cyclin E stabilization.

Inactivation of the GSK3 β –FBW7 Axis Increases DR5 Receptor Levels and Stimulates DR5-Dependent Caspase-8 Processing. Previously, we showed that ectopic expression of MYC induces the up-regulation of DR5 receptor on the cell surface and subsequent sensitization to apoptosis induced by DR5 agonists (5). Thus, we asked whether the additional stabilization of MYC caused by FBW7 or GSK3 β depletion could further influence surface DR5 receptor levels. Transfection of HA1E-MYC cells with siRNAs against *GSK3 β* and *FBW7* resulted in increased levels of DR5 receptor relative to cells transfected with control or *GSK3 α* siRNAs (Fig. 6a).

In contrast, an siRNA targeting *DR5* decreased cell surface DR5 expression, confirming the specificity of the reagents used in these assays. Furthermore, increased DR5 receptor levels in GSK3 β - or

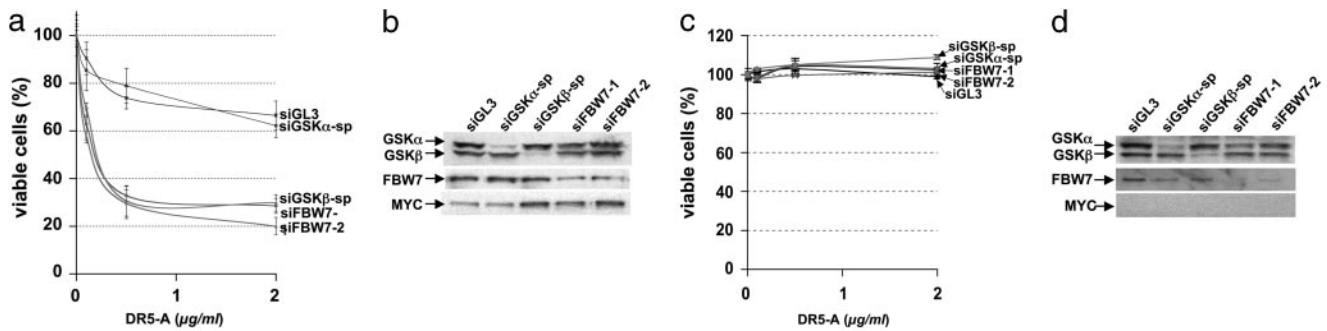


Fig. 4. The GSK3 β -FBW7 MYC degradation pathway determines sensitivity to DR5-A-induced apoptosis. (a and c) HA1E-MYC (a) and HA1E (c) cells were transfected with one of two nonoverlapping siRNAs targeting the MYC ubiquitination component FBW7 (siFBW7-1 or siFBW7-2) and assayed for sensitivity to DR5-A. (b and d) siRNA efficacy in HA1E-MYC (b) and HA1E (d) cells was determined by Western blot analysis. Note that depletion of FBW7 or GSK3 β , but not GSK3 α , results in increased MYC protein levels in only HA1E-MYC cells.

FBW7-depleted cells were associated with increased activation and proteolytic processing of caspase-8 upon DR5 stimulation (Fig. 6b), thus correlating increased DR5 receptor levels to increased functional apoptotic signaling. GSK3 β and FBW7 siRNAs had no detectable influence on DR5 receptors in HA1E cells (data not shown), suggesting that disrupting the GSK3 β /FBW7-dependent MYC degradation process in cells with low MYC expression is not sufficient to enhance DR5 cell surface expression and subsequent sensitization to DR5 agonists.

Similar increases in DR5 surface levels were observed in HCT116 cancer cells upon transfection with GSK3 β and FBW7 siRNAs (Fig. 6c). Furthermore, incrementally reducing FBW7 gene dosage by heterozygous or homozygous mutation in HCT116 resulted in increasing DR5 cell surface levels with decreasing FBW7 gene dosage (Fig. 6d and e). Notably, in HCT116 FBW7^{+/+} and FBW7^{+/-} cells, DR5 cell surface expression could be further increased by transfection of siRNAs against GSK3 β or FBW7 (Fig. 6c and d). However, no further increases could be observed after introduction of these siRNAs into HCT116 FBW7^{-/-} cells devoid of functional FBW7 (Fig. 6e). These results demonstrate that GSK3 β regulates DR5 receptor levels only in the presence of FBW7 and, thus, that GSK3 β acts in a linear pathway with FBW7 to regulate DR5 receptor levels.

Although treatment with the GSK3 inhibitor LiCl or with siRNAs against GSK3 β or FBW7 sensitized HCT116 cells to DR5-A, they had little effect on sensitivity to an agonistic antibody against the DR4 TRAIL receptor (Fig. 6, compare f and g). In

contrast, treatment with the general enhancer of death receptor-induced apoptosis, cycloheximide, or with an siRNA against FLIP (a gene that inhibits caspase-8 activity and, thus, attenuates the DR4 and DR5 signaling pathways) sensitized cells similarly to DR4 or DR5 agonistic antibodies. These data suggest that the sensitization to apoptosis provoked by inactivation of the GSK3 β -FBW7 axis is highly specific for DR5 apoptotic signaling and likely occurs at least in part at the level of DR5 itself, although other mechanisms may also play a role [e.g., see Ricci *et al.* (10)].

The GSK3 β -FBW7 Axis as a Potential Therapeutic Target. Our data suggest that targeting the GSK3-FBW7 axis in MYC-expressing cells might be an effective therapeutic strategy in combination with DR5 agonists. To test this idea *in vivo*, we grew HCT116 FBW7^{+/+} and FBW7^{+/-} cells as s.c. xenografted tumors in nude mice. HCT116 FBW7^{-/-} cells grew poorly *in vivo* and were excluded from the study. When tumors reached a mean volume of ≈ 80 mm³, tumor-bearing mice were treated with 100 μ g of DR5-A every other day for a total of seven treatments or with buffer only as a control. Upon completion of the treatment regimen, the FBW7^{+/-} tumors exhibited a significantly greater response to DR5-A (Fig. 7), with a mean tumor volume of 88 mm³ versus 223 mm³ for the HCT116 FBW7^{+/+} tumors ($P = 0.0087$), suggesting that targeting GSK3 or FBW7 may be effective as a combination therapy with DR5 agonists.

Although we were not able to completely eradicate the FBW7^{+/-} tumors, it should be noted that the increased treatment efficacy

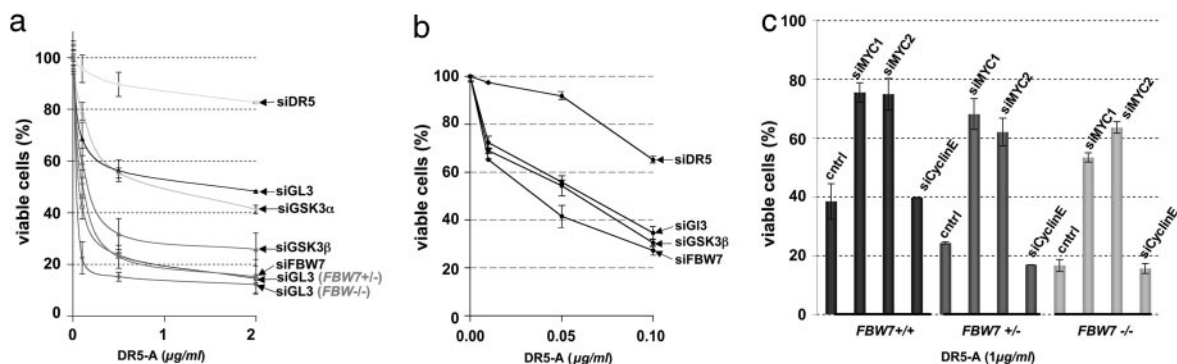


Fig. 5. Depletion or mutation of the FBW7 tumor suppressor enhances DR5-A sensitivity in tumor-derived cell lines in a MYC-dependent manner. (a) The colon carcinoma-derived cell line, HCT116, was transfected with the indicated siRNAs as previously described, and sensitivity to DR5-A was compared with genetically engineered HCT116 cells (see *Methods*) heterozygous (+/-) or homozygous (-/-) for FBW7. Confirmation of the efficacy of siRNA-mediated knockdown and of FBW7 genotypic status is shown in Fig. 9. (b) HCT116 FBW7^{-/-} cells were transfected with siRNAs and analyzed as in a with lower DR5-A concentrations (see Fig. 12 for comparison with higher DR5-A concentrations). (c) HCT116 FBW7^{+/+}, FBW7^{+/-}, and FBW7^{-/-} cells were transfected with control siRNAs (siGL3), two distinct nonoverlapping siRNAs targeting MYC (siMYC1 and siMYC2), and an siRNA specific for cyclin E (siCyclinE). After 48 h, transfected cells were treated with DR5-A for 20 h, and cell viability was determined.

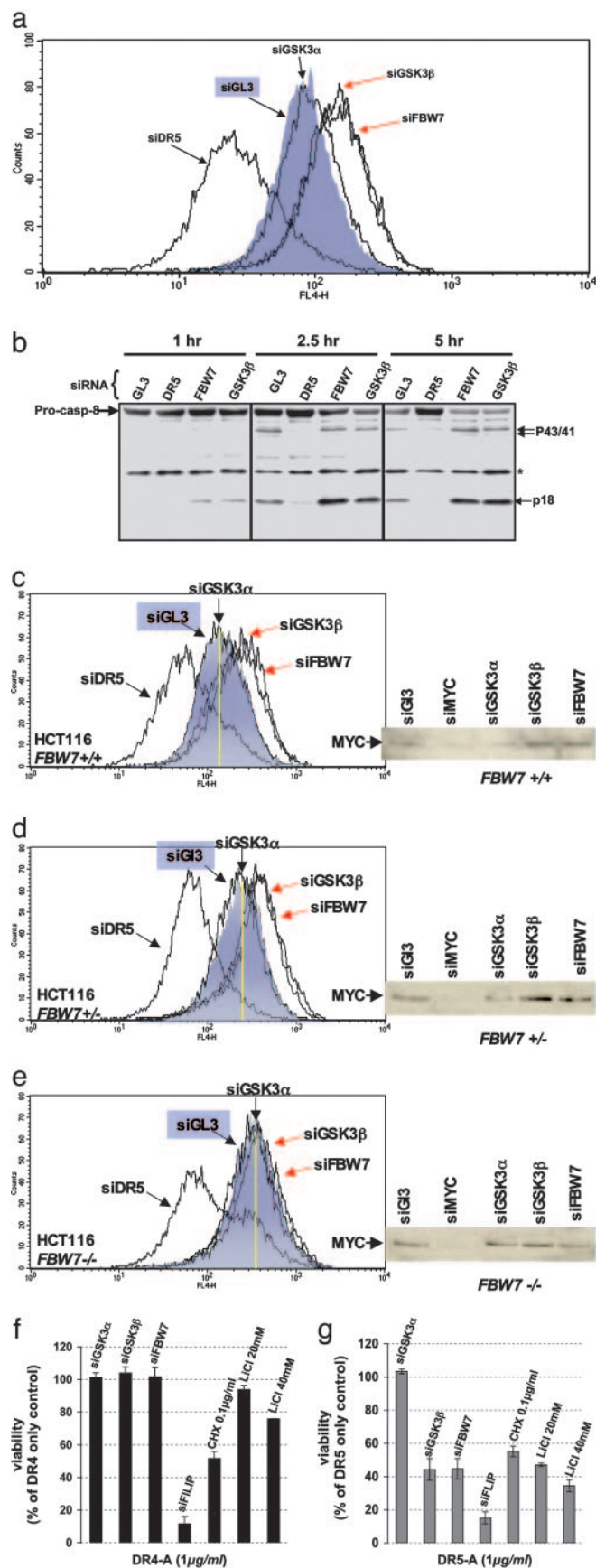


Fig. 6. Inactivation of the GSK3 β -FBW7 axis increases DR5 receptor levels and stimulates DR5-dependent caspase-8 processing in MYC-expressing cells.

observed for the *FBW7*^{+/-} tumors was a result of only a 50% reduction in *FBW7* gene dosage. Furthermore, the HCT116 cell line is known to be defective in mismatch repair and to rapidly evolve resistance to TRAIL pathway agonists *in vivo* because of the acquisition of frameshift mutations in the *BAX* gene [LeBlanc *et al.* (29) and our unpublished data]. Nevertheless, depletion of FBW7, *in vitro* and *in vivo*, enhanced sensitivity to DR5 agonists. Because restoration of FBW7 tumor suppressor function may not be therapeutically feasible, our data may highlight an alternative strategy of exploiting enhanced sensitivity to DR5 agonists for targeting cancer cells with defective FBW7 function.

Discussion

The development of large-scale libraries of siRNAs directed against significant subsets of the genome (30–32) or against biologically interesting and therapeutically relevant gene families (8, 33) has ushered in an era in which the functions of large numbers of genes can be systematically interrogated based on their loss-of-function phenotypes. We and others (34–37) have proposed that one particularly rewarding application of this technology may be in identifying synthetic lethal interactions with genes known to play central roles in cancer cell proliferation or survival. Here we demonstrate the potential of such synthetic lethal screens to identify cancer drug targets, such as *GSK3 β* and *FBW7*, a pair of genes mediating the ubiquitin-dependent degradation of MYC, as genes whose inactivation potentiates apoptosis specifically in MYC-overexpressing cells.

Implications for Cancer Therapy. The sensitization to DR5-mediated apoptosis resulting from suppression of the GSK3 β -FBW7 axis has important implications for how DR5 agonists might be used in the clinic. Mutations at the T58 GSK3 β phosphorylation site on MYC are frequently observed in Burkitt's lymphoma (38) and have been shown to inhibit its degradation by the proteasome (39), whereas inactivating *FBW7* mutations have been reported in a variety of solid tumor types, including breast, ovarian, endometrial, pancreatic, and colorectal carcinomas (23, 40, 41). Such mutations can clearly act as determinants of the response to DR5-A treatment when experimentally introduced into cells (Figs. 3*a* and 5*a*). Thus, it will be of interest to determine whether their presence can be used to prospectively identify those patients who might benefit most from DR5 agonist treatment.

Small-molecule GSK3 inhibitors are currently under development for several indications, including diabetes, neurodegenerative diseases, and bipolar disorder (15). By indirectly blocking MYC degradation, such inhibitors may be safe (see below) and effective against MYC-overexpressing cancers, particularly when combined

(*a*) DR5 cell surface expression was measured by flow cytometry in HA1E-MYC cells 48 h after siRNA transfection with the denoted siRNAs. (*b*) Caspase-8 activation was monitored by proteolytic processing of the caspase-8 zymogen [pro-form, arrow labeled Pro-casp-8 (p55/53); activated form, arrow labeled p18; transient p43/41 form, arrow labeled p43/41]. *, nonspecific band. (*c*–*e*) HCT116 *FBW7*^{+/+} (*c*), *FBW7*^{+/-} (*d*), and *FBW7*^{-/-} (*e*) cells were examined for cell surface DR5 levels by FACS analysis. For comparison, each cell line was transfected with siRNAs specific for *DR5*, *GL3* (control shaded in blue), *GSK3 α* , *GSK3 β* , and *FBW7*. (*Right*) Correlation to MYC protein levels. (*Left*) The yellow line in each FACS panel denotes the control levels of DR5 receptor, which increases with decreasing *FBW7* gene dosage. (*f* and *g*) *FBW7* or *GSK3 β* depletion specifically enhances apoptosis induced through DR5 (*f*) but not DR4 (*g*). Apoptosis was induced in siRNA-transfected or compound-treated HA1E-MYC cells of an agonistic antibody against DR4 (*f*) or DR5 (*g*). Note that treatments disrupting the GSK3 β -FBW7 axis sensitize specifically to DR5-A, whereas depletion of FLIP or treatment with the translation inhibitor cycloheximide (CHX) sensitize to DR4- and DR5-mediated apoptosis. Data were normalized to antibody-treated cells transfected with a control siRNA (siGL3). Treatment with the DR4 and DR5 antibodies alone resulted in \approx 5% and \approx 30% reduction in viability under the conditions used for these assays.

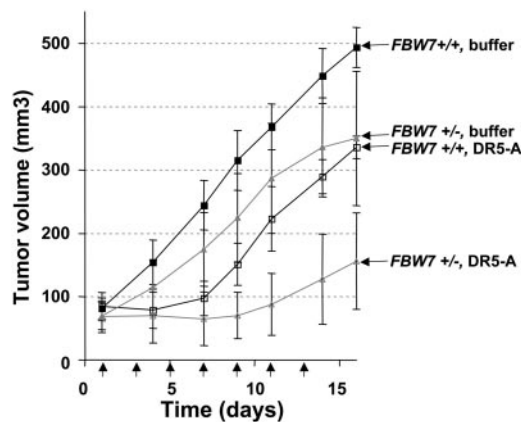


Fig. 7. Response of *FBW7*^{+/+} and *FBW7*^{+/-} tumor xenografts to DR5-A therapy *in vivo*. HCT116 *FBW7*^{+/+} or *FBW7*^{+/-} cells ($n = 3 \times 10^6$ each) were implanted in the flank of BALB/c nude mice (see *Methods*). At a tumor volume of 80 mm³, four animals per group were i.p. injected with 100 μ g of DR5-A or buffer every other day for a total of seven treatments. Tumor sizes were measured with calipers in three dimensions. Arrows indicate the days of treatment. Error bars represent standard deviations.

with DR5 agonists or other therapeutics that may act at least in part by harnessing TRAIL signaling pathways, such as histone deacetylase inhibitors, interferons, retinoids, and arsenic trioxide (42–46). Similarly, direct chemical inhibition of FBW7 functional activity may also be effective, although, to date, small-molecule antagonists of SCF ubiquitin ligase complexes, although currently under investigation, have yet to be clinically tested.

Employing Synthetic Lethality to Elucidate Tumor-Biased Cell Death Pathways. A synthetic lethal relationship between two genes frequently reflects an underlying biochemical interaction between the

proteins encoded by the genes (12). Thus, in uncovering a genetic relationship between *GSK3 β* and *MYC*, we identified *MYC* as a critical substrate mediating *GSK3 β* 's poorly understood cell survival function. Combining this knowledge with previous work from several laboratories, including our own (5, 18, 19, 21, 22), we propose a model for how *GSK3 β* might regulate DR5-mediated apoptosis under conditions in which DR5 agonist levels are limiting or in weakly sensitive *MYC*-positive tumor cells. By phosphorylating *MYC* at T58, *GSK3 β* targets *MYC* for FBW7-dependent ubiquitination and degradation, thereby keeping *MYC* levels low and cell surface DR5 levels below a threshold necessary for activation. In this model, the *GSK3 β* –FBW7 axis can be thought of as a buffer that restrains the phenotypic consequences of *MYC* activation (12). Disruption of this buffering system in *MYC*-expressing cells, either by mutation or pharmacological intervention, thus unleashes *MYC*'s effects, which in the presence of DR5 agonists, results in the catastrophic induction of apoptosis.

The idea that oncogenes might be exploited to suppress tumorigenesis, although unusual, is not without precedent. Like *MYC*, the oncogene *E2F1* is capable of strongly promoting proliferation and apoptosis (47, 48). Surprisingly, *E2F1*-null mice develop a wide range of tumors not seen in similarly aged WT mice (49), suggesting that, under some circumstances, *E2F1*'s apoptotic functions become critical for restraining tumor growth (50). This finding has led to the proposal that *E2F1* can act either as a tumor-suppressor gene or as an oncogene, depending on context. Because of its essential role in proliferation, *MYC* does not act as a classical tumor-suppressor gene; its inactivation results in hypoproliferation rather than predisposition to cancer (51). Nonetheless, our results show that *MYC*'s apoptotic function can be manipulated, e.g., by inhibiting *GSK3 β* /FBW7 function in the presence of DR5 agonists, such that its proapoptotic functions outweigh its proliferative ones, resulting in tumor suppression rather than promotion.

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