The inflammation-associated cytokine interleukin-6 (IL-6) can contribute to tumor growth and resistance to therapy by the activation of survival mechanisms. In several human cancers, IL-6-activated survival signaling involves the signal transducers and activators of transcription (Stat) factors or protein kinase cascades. microRNAs (miRNAs) are endogenous regulators of gene expression that are altered in expression in many cancers. However, the effect of inflammatory cytokines on miRNA expression and the role of miRNA in modulating IL-6-mediated cell survival are unknown. We investigated the involvement of miRNA in malignant cholangiocytes stably transfected to over-express IL-6, which enhances tumor growth in vivo by inhibition of apoptosis. We provide evidence that (i) miRNA expression both in vitro and in vivo is altered by overexpression of IL-6; (ii) selective miRNAs including let-7a are up-regulated and contribute to the survival effects of enforced IL-6 activity; and (iii) let-7a contributes to the constitutively increased phosphorylation of Stat-3 by a mechanism involving the neurofibromatosis 2 (NF2) gene. These findings reveal a novel mechanism by which IL-6 mediates tumor cell survival that may be therapeutically targeted and emphasize the presence of complex interrelationships between deregulated expression of miRNA and transcription factors in human cancers.

Increased expression of the inflammation-associated cytokine interleukin-6 (IL-6) occurs in chronic inflammatory conditions and in several human cancers such as multiple myeloma, prostate cancer, and cholangiocarcinoma. IL-6 has been implicated in tumor growth in many of these tumors, and elevated IL-6 expression has been associated with poor outcomes and resistance to chemotherapy (1). Experimentally, growth of prostate cancer and cholangiocarcinoma xenografts in athymic mice has been shown to be increased by enforced expression of IL-6 by activation of cell survival signaling (2, 3). The mechanisms by which IL-6 promotes cell survival in cancers are of considerable interest because they may be therapeutically targeted.

IL-6-activated survival signaling has been shown to involve the signal transducers and activators of transcription (Stat) factors or various protein kinase cascades (4, 5). Although constitutive activation of Stat has been described in many cancers, the precise mechanisms involved are incompletely understood. Cholangiocarcinomas are highly resistant to chemotherapy. However, inhibition of IL-6-dependent pathways such as the Jak-Stat pathway, phosphatidylinositol 3-kinase, or the p38 MAPK pathways can enhance chemotherapy-induced cell death. Thus, aberrant IL-6-dependent survival signaling may contribute to the refractoriness of cholangiocarcinoma to most chemotherapeutic agents.

We sought to understand the role of microRNAs (miRNAs) in IL-6-mediated tumor cell survival. miRNAs are endogenous regulators of gene expression that are altered in expression in many cancers (6-8). The expression of several miRNAs in cholangiocarcinoma xenografts in athymic mice is altered during in vivo treatment with gemcitabine (9). miRNAs are small endogenous molecules that can regulate gene expression in a sequence complementary manner. Several hundred miRNAs have been identified, and details of the mechanisms by which they regulate gene expression are being unraveled (10, 11). Less is known about the mechanism by which miRNAs contribute to cellular behavior and function. Alterations in miRNA expression occur in many different cancers (8). Thus, individual miRNAs may play contributory or regulatory roles in tumor cell pathogenesis or behavior. Potential downstream targets of miRNA include oncogenes or tumor suppressor genes, but few miRNA-regulated targets relevant to tumor biology have been described such as the Ras oncogene (12). We postulated that genetic reprogramming resulting from altered miRNA regulatory networks may contribute to tumor cell response and resistance to chemotherapy.

**EXPERIMENTAL PROCEDURES**

**Cell Lines and Cultures**—Mz-1 and KMCH-1 human malignant cholangiocytes and their respective IL-6-overexpressing stable transfectants, Mz-IL-6 and KM-IL-6, were obtained and
cultured as described previously (2). Basal IL-6 expression was increased by ~1.5-fold in KM-IL-6 and ~3-fold in Mz-IL-6 cells relative to their respective controls.

Transfections—20 μl of 100 nm microRNA precursor, antisense inhibitor, or controls were added to 1 × 10^6 cells suspended in 80 μl of Nucleofector solution (Amaza Biosystems, Kolin, Germany) at room temperature. Electroporation was performed using the Nucleofector system (Amaza Biosystems). Transfected cells were then resuspended in regular culture medium containing 10% serum for 48–72 h prior to study.

MicroRNA Isolation and Expression Profiling—miRNA was isolated by PAGE purification of total RNA, and expression profiling was performed using a custom-generated microarray as described previously (9). Microarrays were scanned using a GenePix 4000A array scanner (Axon Instruments, Union City, CA). Normalization was performed by expressing each miRNA replicate relative to a control miRNA (Ambion, Austin, TX) added to each sample, thus allowing comparisons between chips. Data were analyzed using GeneSpring 7.0 Software (Silicon Genetics, Redwood City, CA), and an average value of the median intensity of each replicate in four groups was generated. MicroRNA expression levels were clustered using a self-organizing tree algorithm using the MultiExperiment Viewer Version 3.1 from The Institute for Genomic Research (13).

Quantitative Real-time PCR—RNA was isolated using the ToTALLY RNA isolation kit (Ambion), and cDNA was generated by reverse transcription using 1 μg of total RNA and the reverse transcription kit (Invitrogen). Mature let-7a miRNA expression was assessed using a TaqMan® human microRNA assay kit (Applied Biosystems, Foster City, CA). Real-time PCR was performed using a MX 3000P® PCR instrument (Stratagene, San Diego, CA).

Cytotoxicity Assay—Cell viability was assessed using a commercially available tetrazolium bio-reduction assay as described previously (14). 10,000 viable cells/well were seeded into 96-well plates and incubated with gemcitabine, 5-fluorouracil, camptothecin, or appropriate diluent controls in a final volume of 200 μl of medium containing 0.5% fetal bovine serum.

Caspase Assay—Cells were plated in 96-well plates (20,000 cells/well) and incubated with different chemotherapeutic agents or diluent control. Caspase 3/7 activity was assayed using the fluorometric Apo-ONE homogenous caspase 3/7 assay (Promega, Madison, WI) and a Cytofluor microplate fluorescence plate reader.

Stat-3 Kinase Assay—Stat-3 activity was assessed in cell lysates after immunoprecipitation using monoclonal P-Stat-3-Tyr705 antibody (Cell Signaling Technology) and using a tyrosine kinase activity assay kit (Chemicon, Temecula, CA).

Luciferase Reporter Vectors—The pMIR-NF2-luc and pMIR-NF2-MUT-luc firefly luciferase reporter vectors, which contain the intact or mutated putative let-7a recognition sequence from the 3’-UTR of NF2, respectively, cloned downstream of the firefly luciferase gene were constructed as follows. Synthetic oligonucleotides encompassing the intact or mutated let-7a recognition sequence, sticky ends for HindIII and SpeI to eliminate digestion of the insert, and a unique BlpI site to test for correct orientation were used to anneal the oligonucleotides. The recognition sequence is italicized in the former sequence, and the random mutations introduced are italicized in the latter sequence. To anneal the oligonucleotides, 2 μg of each strand was added to 46 μl of DNA annealing buffer (30 mM HEPES, pH 7.4, 100 mM potassium acetate, 2 mM magnesium acetate) for a final volume of 50 μl and incubated at 90 °C for 3 min and then at 37 °C for 1 h. The annealed insert was then directly ligated into the HindIII and SpeI cloning sites of the pMIR-REPORT luciferase expression vector (Ambion).
Clones were selected after screening by restriction digestion with BlpI. Mz-IL-6 cells were co-transfected with 1/μg of pMIR-NF2-UTR or pMIR-NF2-MUT-UTR construct and 1/μg of pRL-TK Renilla luciferase expression construct without (empty vector control) or with anti-let-7a inhibitor using TransIT-siQUEST transfection reagent (Mirus, Madison, WI). Luciferase assays were performed 48 h after transfection using the Dual-Luciferase reporter assay system (Promega). For validation of the effect of anti-let-7a, we used the pRL-TK let-7a firefly luciferase expression construct in which two let-7a sites are inserted into the XbaI site in the 3’-UTR. Cells were co-transfected with pRL-TK, and luciferase assays were performed 48 h after transfection. Firefly luciferase activity was normalized to Renilla luciferase activity for each sample.

Western Blotting—For immunoblot analysis of cells in culture, cell lysates were obtained from cells grown in 100-mm dishes, whereas for analysis of xenograft tissue, lysates were obtained after tissue homogenization. Equivalent amounts of protein were resolved by electrophoresis in a 4–20% Tris-HCl gel (Bio-Rad) and then transferred to nitrocellulose membranes. After blocking, membranes were incubated with primary antibodies and infrared dye-labeled secondary antibodies. The protein of interest was then detected using the LI-COR Odyssey infrared imaging system (LI-COR Bioscience, Lincoln, NE). Nuclear and cytoplasmic fractions were obtained using the NE-PER extraction kit (Pierce) according to the manufacturer’s instructions.

Xenograft Model—Studies were performed under an Institutional Animal Care and Use Committee (IACUC) approved protocol. Eight-week-old male athymic nu/nu mice (Charles River Laboratories, Wilmington, MA) were maintained in accordance with IACUC procedures and guidelines. 5 × 10⁶ Mz-1 or Mz-IL-6 cells were suspended in 0.25 ml of extracellular matrix gel, and the mixture was injected subcutaneously into the right and left flanks. Serial measurements of xenograft growth were performed, and tumor volume was estimated using the formula 4/3π(L*W*H/8). Once tumor volume was 200–230 mm³, xenografts were excised, and tissue was homogenized for miRNA isolation or immunoblot studies or used for miRNA inhibition studies. For the latter, mice with Mz-IL-6 xenografts were injected intratumorally with 4 ng/mm³ tumor volume of either anti-let-7a or diluent. The following day, gemcitabine (150 mg/kg) was administered intraperitoneally every 3 days for three doses. The change in tumor size was assessed, and tumors were excised after 10 days. Homogenates were obtained for Western blot analysis. Sections of tumors were obtained for immunofluorescence studies using mouse anti-P-Stat-3 (Tyr705) (1:75 dilution) or rabbit
**RESULTS**

**IL-6 Survival Signaling Involves Alterations in miRNA Expression**—To identify miRNA that may contribute to survival signaling and chemoresistance, we first assessed the effect of IL-6 on miRNA expression. Mz-CHA-1 human cholangiocarcinoma cells were stably transfected to overexpress IL-6 (Mz-IL-6 cells) and implanted as xenografts in athymic nude mice. When compared with Mz-1 control cell xenografts, the growth rate of Mz-IL-6 xenografts was increased (Fig. 1A). Moreover, there was a loss of sensitivity of Mz-IL-6 tumor xenografts to the chemotherapeutic agent gemcitabine (Fig. 1B), in conjunction with a decrease in the number of TUNEL-positive (apoptotic) cells when compared with controls (Fig. 1C). We used a miRNA microarray to assess the expression of human miRNAs in tumor cell xenografts and in two different cholangiocarcinoma cell lines overexpressing IL-6. The pattern of miRNA expression in IL-6-overexpressing Mz-IL-6 and KM-IL-6 cells differed from their controls (supplemental Fig. 1). A cluster of miRNAs that were increased with enforced IL-6 expression both in vitro as well as in vivo was identified and included several members of the let-7 family and miRNA that have been implicated in oncogenesis such as miR-21 (Fig. 2A) (17). These data showing altered miRNA expression profiles in vivo suggest that some effects of IL-6 on tumor cell growth and apoptosis may be mediated by miRNA-dependent regulation of gene expression. The relative expression of several members of the let-7 family was altered in vivo (Fig. 2B). Of these, let-7a was chosen for further study based on consistency and level of expression. The expression of mature let-7a miRNA was confirmed to be increased by real-time PCR in IL-6-overexpressing cells in vitro, as well as in tumor cell xenografts in vivo when compared with controls (Fig. 2C).

**let-7a Contributes to Survival Signaling by IL-6**—To determine the relevance of enhanced let-7a expression to survival signaling, we next evaluated the effect of let-7a inhibition on the response to chemotherapy in vitro. The effect of the anti-let-7a inhibitor was assessed by co-transfecting with the pRL-Tk let-7a firefly luciferase construct that contains two let-7a sites in the 3'-UTR of the firefly luciferase reporter. An increase in luciferase activity confirmed the efficacy of anti-let-7a under...
the conditions used for our studies (Fig. 3A). Cytotoxicity in response to diverse agents was enhanced by preincubation with antisense inhibitors to let-7a (Fig. 3B). Moreover, there was an increase in caspase-3/7 activity in response to chemotherapy in cells preincubated with anti-let-7a when compared with a control inhibitor (Fig. 3C). Similarly, an increase in PARP cleavage and activated caspase-3 was noted in response to anti-let-7a in Western blots from gemcitabine-treated cells (Fig. 3D). Thus, inhibition of let-7a increases chemotherapy-induced apoptosis. Taken together, these data are consistent with an effect of let-7a on IL-6-mediated anti-apoptotic survival pathways.

let-7a Regulates Stat-3 Phosphorylation—Survival signaling by IL-6 can involve activation of the Stat family of transcription factors or protein kinases such as p38 MAPK and phosphatidylinositol 3-kinase. Constitutive phosphorylation and activation of Stat-3 in cholangiocarcinoma cells have been shown to be IL-6-dependent (18). Consistent with these observations, there was an increase in caspase-3/7 activity in response to chemotherapy in cells preincubated with anti-let-7a when compared with a control inhibitor (Fig. 3C). Similarly, an increase in PARP cleavage and activated caspase-3 was noted in response to anti-let-7a in Western blots from gemcitabine-treated cells (Fig. 3D). Thus, inhibition of let-7a increases chemotherapy-induced apoptosis. Taken together, these data are consistent with an effect of let-7a on IL-6-mediated anti-apoptotic survival pathways.

**FIGURE 4.** Overexpression of IL-6 increases constitutive STAT-3 activation. A, IL-6-overexpressing Mz-IL-6 or control Mz-1 cells were transfected with negative control miRNA inhibitors or with miR-21- or let-7a miRNA-specific inhibitors for 48 h. The expression of active Stat-3, Akt, and p38 MAPK was assessed by Western blot analysis using active site phosphorylation-specific antibodies to evaluate IL-6-activated survival signaling pathways. P-STAT3, phosphorylated Stat-3; P-Akt, phosphorylated Akt; P-p38, phosphorylated p38. B, let-7a modulates expression of activated Stat-3 and its downstream anti-apoptotic targets Mcl-1 and survivin in vitro. Cells were transfected with anti-let-7a or negative control miRNA inhibitors in vitro, and cell lysates were obtained after 48 h. The expression of survivin and Mcl-1 is increased in Mz-IL-6 cells when compared with Mz-1 cells, and moreover, it is decreased by either anti-let-7a or AG490, an inhibitor of Jak-mediated STAT-3 activation.

**FIGURE 5.** NF2 is a target of let-7a. A, the location of the putative let-7a target site in the NF2 3′-UTR is shown. A comparison of base pairs between mature human let-7a (hsa-let-7a), human NF2, rat nro-let-7a, and rat NF2 shows sequence conservation between species. The sequence of the mutated target site with mutations to disrupt base pairing between let-7a binding sites and NF2 is also shown. B, Mz-IL-6 cells were transfected with the Renilla luciferase expression construct pRL-tk and either the luciferase construct pMIR-NF2-luc or pMIR-NF2-MUT-luc (in which mutations were introduced in the let-7a target site) with either anti-let-7a or control inhibitor. After 48 h, Dual-Luciferase assays were performed. An increase in relative firefly luciferase with pMIR-NF2-luc (black bars) but not with the pMIR-NF2-MUT-luc construct (gray bars) confirms that the let-7a complementary sequence in the 3′-UTR of NF2 is a target of modulation by let-7a. The data represent the mean and standard deviations from six determinations from three independent transfections. *, p < 0.05 relative to respective controls.
NF2 Is a Target for let-7a

NF2, a regulator of STAT-3 activation, was decreased in Mz-IL-6 cells in a let-7a-dependent manner. Anti-let-7a increases NF2 expression in Mz-IL-6 cells concomitant with a decrease in Tyr705 phospho-Stat-3. Quantitative data showing the mean and 95% confidence interval from four separate studies are shown. B, Mz-1 cells were transfected with siRNA to NF2 or scrambled control siRNA. When compared with control siRNA transfected cells, the phosphorylation of Stat-3 was increased by NF2 siRNA. C, Mz-1 cells were incubated with either let-7a precursor or siRNA to NF2 for 48 h. Nuclear and cytoplasmic fractions were obtained, and immunoblots were performed for Stat-3 expression. TATA binding protein and β-actin were used as nuclear and cytoplasmic markers, respectively, and as loading controls. An increase in nuclear Stat-3 occurs with either let-7a precursor or with siRNA to NF2.

in chemoresistance, the expression of both survivin and Mcl-1 was increased in Mz-IL-6 cells in a let-7a- and Stat-3-dependent manner (Fig. 4B).

NF2 Is a Target for let-7a

To elucidate potential mediators of let-7a modulation of Stat-3 phosphorylation, we performed a bioinformatics analysis. The let-7a sequence was compared with proposed regulators of Stat-3, using the criteria of Doench and Sharp (15) but modified to include 85% sequence complementarity at positions 2–9 of the miRNA. The tumor suppressor gene NF2, a known regulator of Stat-3 activation, was identified as a putative target for let-7a. Moreover, interrogation of various target prediction databases such as TargetScan, miRScan, miRanda, and PicTar did not identify any other known regulators of Stat-3 as potential targets for let-7a (21). The location of the let-7a complementary site in the 3′-UTR of NF2 is shown in Fig. 5. The site is conserved in human and rat homologs of NF2.

NF2 has been previously shown to regulate Stat-3 phosphorylation by a mechanism involving the hepatocyte growth factor tyrosine kinase substrate HRS (22). We verified that NF2 was a target for let-7a using luciferase reporter constructs containing the let-7a recognition sequence from the 3′-UTR of NF2 inserted downstream of the luciferase gene (pMiR-NF2-luc), along with a similar construct in which random mutations were introduced at sites involved in base-pairing (pMiR-NF2-MUT-luc) (Fig. 5A). Transfection with anti-let-7a increased reporter activity in Mz-IL-6 cells, whereas let-7a precursor decreased reporter activity in Mz-1 cells. However, these effects were ameliorated when the mutated reporter construct pMiR-NF2-MUT-luc was used in place of pMiR-NF2-luc (Fig. 5, B and C). Constitutive expression of NF2 was decreased and p-Stat-3 increased in Mz-IL-6 cells when compared with Mz-1 controls. Moreover, inhibition of let-7a increased NF2 expression and concomitantly decreased p-Stat-3 (Fig. 6A). Conversely, p-Stat-3 was increased during incubation of Mz-1 cells with NF2 siRNA (Fig. 6B). Incubation with the let-7a precursor miRNA increased nuclear Stat-3 expression, suggesting that let-7a enhances activation of Stat-3 and nuclear translocation. Similar effects were also observed with NF2 siRNA (Fig. 6C). These studies support a mechanism by which enhanced IL-6 production enhances constitutive Stat-3 phosphorylation and activation via a mechanism involving let-7a-mediated inhibition of NF2.

let-7a Can Mediate Downstream Effects of IL-6 Overexpression on Stat-3 Phosphorylation

To explore the in vivo relevance of these observations, we assessed the expression of p-Stat-3, NF2, and the Stat-3-regulated anti-apoptotic proteins in xenografts from xenografts. The results corresponded to those observed in vitro with an increase in basal expression of p-Stat-3 and decrease in NF2 in Mz-IL-6 xenografts when compared with control xenografts (Fig. 8A). Intratumoral administration of anti-let-7a increased NF2 and decreased p-Stat-3 expression in Mz-IL-6 xenografts in vivo (Fig. 8, B and C). Moreover, a decrease in tumor growth consistent with increased gemcitabine toxicity was observed in response to anti-let-7a when compared with tumors that were
untreated. Anti-let-7a (n = 6) or diluent (n = 4) was adminis-
tered intratumorally into Mz-IL-6 xenografts. Animals subse-
quently received a course of three doses of gemcitabine 150
mg/kg intraperitoneally given every 3 days. The mean change in
tumor growth at the end of treatment was 0.6 ± 2.0% in tumors
that received anti-let-7a when compared with 18.4 ± 8.2% in
controls (p = 0.02). In combination, these findings identify a
previously unrecognized mechanism that contributes to con-
stitutive Stat-3 phosphorylation involving NF2, a target of reg-
ulation by the let-7a microRNA.

DISCUSSION

Although the association between chronic inflammation and
malignancy has been recognized for many decades, the role of
miRNA in cancer cell biology has only recently been appreci-
ated. The role of cytokines as stimulators of miRNA expression
have not been explored, and here, we demonstrate a role for
persistent IL-6 stimulation on altered miRNA expression in a
human cancer. The demonstration of an inflammation-associ-
ated cytokine-regulated miRNA-mediated survival mechanism
is highly relevant to both tumor biology and regulation of cyto-
kine signaling. Moreover, these studies emphasize the emerg-
ing complexity of miRNA-mediated cellular responses.

There is compelling evidence of a critical role for activated
Stat-3 in human cancers. Constitutively activated Stat-3 is
observed in many cancers, and abrogation of Stat-3 activation
results in the loss of the malignant phenotype (23). Moreover,
cells expressing persistently activated Stat-3 are dependent on
it for survival. Thus, Stat-3 can act as an oncogene and may
contribute to tumor growth (24–26). Although several cyto-
kines including IL-6 can induce Stat-3 tyrosine phosphoryla-
tion, the mechanisms by which it is constitutively activated in
cancers are unknown. Although activating Stat-3 mutations
have not been described, aberrant expression of modulators of
Stat expression or phosphorylation such as PIAS-3, modulators
of upstream Stat-3 activation such as SOCS-1, or as yet unchar-
acterized Stat-3 tyrosine phosphatases may all contribute (5).
None of these mechanisms have been shown to predominate in
tumor cells. The contribution of miRNA modulation of NF2 expression warrants further investigation as an alternative mechanism contributing to constitutive Stat-3 activation. We speculate that the NF2-dependent mechanism may be more relevant to constitutively increased Stat-3 phosphorylation in the setting of chronic IL-6 stimulation, rather than transient, non-sustained activation of Stat-3 in response to acute stimulation of IL-6 signaling, which has been well characterized and involves, among others, Jak-Stat interactions.

NF2 is located on chromosome 22q12.2 and encodes for merlin, a putative tumor suppressor gene. Merlin has strong binding to HRS, a potent regulator of receptor tyrosine kinase trafficking, and the interaction of HRS and Merlin can result in inhibition of Stat activation (22). Merlin has shown to act as growth regulator, and its decreased expression could partly contribute to the increased growth rate observed in IL-6-overexpressing tumor cell xenografts. In response to IL-6 stimulation, activation of Stat-3 is associated with the endocytotic pathway (27). Thus, a plausible mechanism by which decreased NF2 expression in response to persistent IL-6 stimulation results in activation of Stat-3 could involve facilitating its association with the endocytotic pathway through an HRS-mediated mechanism.

The miRNA family of let-7 and its homologs has been implicated as cancer-associated miRNAs in recent studies (28, 29). Although we focused our studies on let-7a, we note that the relative expression of other members of the let-7 family such as let-7d and let-7f-2 were also increased in vivo (Fig. 2B). It is quite likely that these other members of the let-7 family that are differentially altered in response to increased IL-6 stimulation may also have cellular actions. NF2 is not a predicted target for either let-7d or let-7f-2. However, both these miRNAs could potentially target SOCS-1, an established inhibitor of the Jak-Stat-3 pathway, and thereby modulate IL-6-dependent Stat-3 activation.

In reported series of lung cancers, let-7 expression is downregulated in association with Ras expression in the setting of activating Ras mutations, and decreased expression of let-7a2 has been shown to correlate with a poor prognosis (12, 30, 31). However, these observations are likely to be cell type-specific since let-7 is only sporadically reduced in tumor types other than lung cancer. Our experimental model differs considerably from these studies in representing a state of persistent cytokine stimulation, and it is unknown whether let-7 expression can be modulated by IL-6 in a similar manner in lung cancer. Although augmenting let-7 expression is being touted as a potential ther-

FIGURE 8. STAT-3 phosphorylation is increased by enforced expression of IL-6 in vivo. A, tumor cell xenografts in nude mice (three for each cell type) were excised once they grew to a volume of ~ 200 mm$^3$ and tissue-homogenized. The expression of Tyr$^{705}$ phosphorylated Stat-3, its negative upstream modulator NF2, and the downstream anti-apoptotic Stat-3 targets survivin and Mcl-1 were assessed by Western blot analysis. Quantitative data of mean and 95% confidence intervals from three separate blots are shown. B, Mz-IL-6 tumor xenografts were treated with anti-let-7a or control miRNA inhibitor, and homogenates were obtained for immunoblot analysis. Representative blots and quantitative data showing the average and 95% confidence interval of four separate blots are shown. C, immunohistochemistry for phosphorylated Stat-3 and NF2 was performed on xenograft sections, showing a decrease in phospho-Stat-3 expression and an increase in NF2 in xenografts injected with anti-let-7a when compared with controls. *, p < 0.05 relative to respective controls.
let-7a Modulates IL-6 Survival Signaling

therapeutic strategy, such approaches may be inappropriate for cancers that are associated with elevated IL-6 levels such as cholangiocarcinoma. In contrast, potential interventions to decrease survival signaling and Stat-3 activation by IL-6 may be a useful approach for these cancers.

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