Signal transducer and activator of transcription 3 (Stat3) is activated in a variety of malignancies, including hepatocellular carcinoma (HCC). Activation of Ras occurs frequently at advanced stages of HCC by aberrant signaling through growth factor receptors or inactivation of effectors negatively regulating Ras signaling. Here, we addressed the role of Stat3 in Ras-dependent HCC progression in the presence and absence of p19ARF/p14ARF. We show that constitutive active (ca) Stat3 is tumor suppressive in Ras-transformed p19ARF−/− hepatocytes, whereas the expression of Stat3 lacking Tyr705 phosphorylation (U-Stat3) enhances tumor formation. Accordingly, Ras-transformed Stat3Dhc/p19ARF−/− hepatocytes (lacking Stat3 and p19ARF) showed increased tumor growth, compared to those expressing Stat3, demonstrating a tumor-suppressor activity of Stat3 in cells lacking p19ARF. Notably, endogenous expression of p19ARF in Ras-transformed hepatocytes conveyed oncogenic Stat3 functions, resulting in augmented or reduced HCC progression after the expression of caStat3 or U-Stat3, respectively. In accord with these data, the knockdown of p14ARF (the human homolog of p19ARF) in Hep3B cells was associated with reduced pY-Stat3 levels during tumor growth to circumvent the tumor-suppressive effect of Stat3. Inhibition of Janus kinases (Jaks) revealed that Jak causes pY-Stat3 activation independently of p14ARF levels, indicating that p14ARF controls the oncogenic function of pY-Stat3 downstream of Jak. Conclusion: These data show evidence that p19ARF/p14ARF determines the pro- or anti-oncogenic activity of U-Stat3 and pY-Stat3 in Ras-dependent HCC progression. (HEPATOLOGY 2011;54:164-172)

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in many human cancers, and (4) Stat3 contributes to abrogated immune surveillance, leading to enhanced tumor cell growth.\(^3-6\) However, recent findings in glioblastoma and intestinal tumors support the idea that Stat3 can also act as a tumor suppressor.\(^7-9\)

In the liver, Stat3 is important for liver regeneration by stimulating hepatic cell proliferation and survival.\(^10\) Stat3 is up-regulated and activated in the vast majority of human hepatocellular carcinoma (HCC) specimens\(^11,12\) and is essential for cell growth, survival, tumor dedifferentiation, intratumoral microvesSEL density, and metastasis of HCC.\(^13,14\) Deactivation of Stat3 by low molecular compounds or inhibition of Stat3 expression by employing RNA-interference approaches enhanced the chemosensitivity of HCC cells and suppressed growth and metastasis of human HCC in xenografted mice.\(^15,16\) Recent findings demonstrate that Stat3 is a critical regulator of liver cancer development and progression through a negative cross-talk with nuclear factor-\(\kappa\)B.\(^17\)

Notably, the constitutive activation of Stat3 is accompanied by high levels of unphosphorylated Stat3 (U-Stat3), which differs from Tyr\(^{705}\) phosphorylated Stat3 (pY-Stat3)-mediated gene expression in both its binding partners and mechanism to activate transcription. The formation of U-Stat3 complexes occurs either in the cytoplasm or the nuclear compartment. Its transcriptional targets also differ from those of pY-Stat3 dimers, as other promoters can be modulated by, for example, U-Stat3/nuclear factor-\(\kappa\)B heterodimers.\(^18\)

The Ras cascade mainly transduces extracellular signals via activated growth factor receptors, resulting in proliferative and anti-apoptotic signals.\(^19\) In HCC, the expression of oncogenic Ras, which is locked in its active form due to the insensitivity against guanosine triphosphatase–activating proteins (GAPs), is rare.\(^19,20\) Yet, aberrant activation of Ras signaling is frequently observed by overexpression of Ras, epigenetic silencing of GAPs by promoter hypermethylation, or by mutations of upstream inducers or downstream effectors.\(^19,20\)

The tumor suppressor p14\(^{\text{ARF}}\) (the human homolog of p19\(^{\text{ARF}}\)) is an important sensor of hyperproliferative stimuli that restricts cell proliferation through both p53-dependent and p53-independent pathways when activated by sustained mitogenic or oncogenic signals, such as Ras.\(^21-23\) Disruption of the p14\(^{\text{ARF}}\)-Mdm2-p53 pathway is a very common feature in cancer.\(^23\) Remarkably, p14\(^{\text{ARF}}\) is inactivated by promoter hypermethylation in up to 40% of HCC cases.\(^23,24\)

In this study, we found that caStat3 acted in a tumor-suppressive manner in Ras-transformed p19\(^{\text{ARF}}\)–/– hepatocytes, whereas the expression of U-Stat3 or loss of Stat3 increased tumor growth. Reciprocal effects of caStat3 and U-Stat3 were observed in Ras-transformed hepatocytes endogenously expressing p19\(^{\text{ARF}}\). In human HCC cells, knockdown of p14\(^{\text{ARF}}\) resulted in reduced pY-Stat3 levels upon tumor formation, thus impeding the tumor-suppressive function of Stat3. Activation of pY-Stat3 by Jak was not affected by p14\(^{\text{ARF}}\) levels, suggesting that p14\(^{\text{ARF}}\) modulates the oncogenic function of Stat3 downstream of Jak in Ras-transformed hepatocytes.

**Materials and Methods**

**Cell Culture.** Mouse hepatocyte cell lines were generated by stable retroviral transmission of immortalized p19\(^{\text{ARF}}\)–/– hepatocytes (MIM-1-4), Stat3\(^{\text{Ahe}/\text{p19ARF–/–}}\), or MMH-D3 cells with a construct expressing oncogenic v-Ha-Ras or Stat3 variants (wtStat3, caStat3, caStat3\(_\beta\), and U-Stat3; Supporting Fig. 1)\(^8\) and cultured on collagen-coated dishes.\(^25,26\) Ras-transformed mouse hepatocytes and human Hep3B, PLC/PRF/5, SW480, and p14\(^{\text{ARF}}\)–deficient MCM1 melanoma cells\(^27\) (a kind gift of Dr. Mario Mikula, Medical University of Vienna) were cultivated at 37°C and 5% CO\(_2\). All cells were routinely screened for the absence of mycoplasma. Details for treatment of HCC cells with cytokines and Jak inhibitors as well as for lentiviral-mediated knockdown of p14\(^{\text{ARF}}\) are provided in the Supporting Information.

**Gene-Targeted Mice.** Stat3\(^{\text{Ahe}}\) mice harboring the liver-specific Stat3 null allele were generated as described.\(^28\) Stat3\(^{\text{Ahe}}\) mice were crossed to p19\(^{\text{ARF}}\)–/– mice to obtain Stat3\(^{\text{Ahe}/\text{p19ARF–/–}}\) mice.\(^29\)

**Isolation and Immortalization of Hepatocytes from Stat3\(^{\text{Ahe}/\text{p19ARF–/–}}\) Mice.** Hepatocytes of 4-week-old Stat3\(^{\text{Ahe}/\text{p19ARF–/–}}\) mice were isolated by liver perfusion and propagated as described.\(^26\) MIM-Stat3\(^{\text{Ahe–/–}}\) and MIM-Stat3\(^{\text{Ahe–/–}}\) cells were obtained by single-cell cloning and employed for retroviral expression of oncogenic v-Ha-Ras. MIM-R-Stat3\(^{\text{Ahe–/–}}\) hepatocytes were used for the stable coexpression of wtStat3, termed MIM-R-Stat3\(^{\text{Ahe–/–} \cdot \text{wtStat3}}\).

**Tumor Formation and Recultivation of Tumor Cells.** Briefly, 1 \(\times 10^6\) murine or 5 \(\times 10^6\) human cells in 100 \(\mu\)L of Ringer solution were subcutaneously (SC) injected into severe combined immunodeficient (SCID) mice (Harlan Laboratories, San Pietro, Italy). Tumor volume was determined as previously described.\(^30\) Pulmonary metastatic colonization was analyzed after the injection of 1 \(\times 10^5\) cells/100 \(\mu\)L of Ringer solution into the tail vein of SCID mice.
Orthotopic liver transplantation was performed by the injection of $1 \times 10^6$ cells/20 µL of Ringer solution into the spleen of SCID mice. Recovery of tumor cells is provided in the Supporting Information. All experiments were performed according to the Austrian guidelines for animal care and protection.

**HCC Induction.** To initiate tumor development in the liver, 14-day-old Stat3$^{fl/fl}$ and Stat3$^{Ahe}$ mice were intraperitoneally (IP) injected with a single dose of diethylnitrosamine (DEN; 25 mg/kg). At 12 months of age, mice were sacrificed and livers were processed for polymerase chain reaction (PCR) analysis or fixed in 4% formaldehyde for immunohistochemistry.

**Immunohistochemistry.** Mice were sacrificed and tumors and lungs were fixed as previously described. Next, 4-µm-thick, paraffin-embedded sections were stained with hematoxylin and eosin (H&E). For immunohistochemistry, sections were stained with anti–phospho-Stat3 (Tyr$^{705}$; Cell Signaling Technology, Beverly, MA) and anti-Stat3 antibodies (Cell Signaling Technology).

**Immunoblotting.** Immunoblotting was performed as described. The primary anti–phospho-Stat3 (Tyr$^{705}$), anti-Stat3 (both from Cell Signaling Technology), anti-p14$^{ARF}$, and anti-actin antibodies (both from Sigma, St. Louis, MO) were used at dilutions of 1:1,000.

**Reverse-Transcription PCR.** Semiquantitative reverse transcription PCR was performed as previously described. Quantitative PCR was performed with Fast SYBR Green Master Mix (Applied Biosystems, Foster City, CA), according to the recommendations of the manufacturer, and quantified with the 7500 Fast Real-Time PCR System (Applied Biosystems). Arbitrary units were calculated by the dCT method. Primer sequences are provided in the Supporting Information.

**Statistical Analysis.** Data are expressed as means ± standard deviation (SD). The statistical significance of differences was evaluated using an unpaired, nonparametric Student $t$ test. Significant differences between experimental groups were $^*P < 0.05$, $^{**}P < 0.01$, or $^{***}P < 0.005$.

**Results**

**Stat3 Represses Tumor Growth of Ras-Transformed p19$^{ARF−/−}$ Hepatocytes.** We employed an established mouse tumor transplantation model to assess the role of Stat3 during HCC progression. This model is based on the lack of p19$^{ARF}$ in hepatocytes, which allows immortalization. Non-tumorigenic p19$^{ARF−/−}$ hepatocytes (MIM-1-4) were transformed with oncogenic Ras (MIM-R). To study the effect of Stat3 on Ras-dependent tumor growth, caStat3 variants of Stat3$^\alpha$ and the natural splice variant, Stat3$^\beta$, lacking the Ser$^{727}$ phosphorylation site and U-Stat3 (lacking both Tyr$^{705}$ and Ser$^{727}$ phosphorylation sites), were stably expressed in MIM-R hepatocytes (Supporting Fig. 1, A,B).

Proliferation kinetics showed no changes between MIM-R hepatocytes and those expressing Stat3 mutants (data not shown). To investigate tumorigenicity, Stat3 mutant hepatocytes were SC injected into SCID mice. MIM-R-caStat3$^\alpha$- and MIM-R-wtStat3-derived tumors displayed two-fold reduced volumes, compared to those generated by MIM-R hepatocytes. An even five-fold suppression of tumor growth was observed upon the injection of MIM-R-caStat3$^\beta$ hepatocytes. On the contrary, MIM-R-U-Stat3 cells caused a two-fold increased tumor volume, compared to MIM-R hepatocytes (Fig. 1A). Orthotopic transplantation of MIM-R-wtStat3, MIM-R-caStat3$^\alpha$, and MIM-R-caStat3$^\beta$ hepatocytes led to a strong reduction of HCC formation, whereas MIM-R-U-Stat3 cells exhibited enhanced HCC generation, compared to MIM-R-derived liver tumors (Fig. 1B). Notably, both frequency and size of lung metastases were significantly reduced after tail vein injection of MIM-R-wtStat3, MIM-R-caStat3$^\alpha$, or MIM-R-caStat3$^\beta$ cells. In contrast, MIM-R-U-Stat3 cells showed pulmonary metastasis comparable to MIM-R hepatocytes (Fig. 1C, D).

These data show that exogenous expression of caStat3 or U-Stat3 causes anti- or pro-oncogenic effects in p19$^{ARF−/−}$ MIM-R hepatocytes, respectively.

**Loss of Stat3 Promotes Tumor Formation in p19$^{ARF−/−}$ MIM-R Hepatocytes.** To verify a tumor-suppressive role of Stat3, we performed a conditional Stat3 knockout in hepatocytes of p19$^{ARF−/−}$ mice. Hepatocytes were isolated from Stat3$^{Ahe}$/p19$^{ARF−/−}$ mice, and deletion of Stat3 was confirmed by PCR and immunoblot analysis (Supporting Fig. 2A, B). Two randomly isolated clones of the hepatocyte pool, designated MIM-Stat3$^{Ahe−1}$ and -2, expressed several hepatocyte-specific markers, such as keratin 18, hepatocyte nuclear factor-1x (HNF-1x), and HNF-4x (Supporting Fig. 2C).

We next analyzed the tumorigenic potential after SC injection into SCID mice. Both, MIM-R-Stat3$^{Ahe−1}$ and MIM-R-Stat3$^{Ahe−2}$ hepatocytes showed increased tumor development, compared to MIM-R cells, whereas the re-expression of wtStat3 in MIM-R-Stat3$^{Ahe}$ hepatocytes abolished faster tumor kinetics (Fig. 2). In summary, these results confirm that Stat3
has tumor-suppressive functions in Ras-transformed p19ARF−/− hepatocytes.

**Stat3 Acts in a Pro-Oncogenic Manner in p19ARF-Positive Ras-Transformed Hepatocytes.** To investigate a possible impact of p19ARF deficiency on Stat3 functions, we employed murine MMH-D3 hepatocytes that express endogenous p19ARF (Fig. 3A). MMH-D3 cells transformed with oncogenic Ras (MMH-R) were further analyzed after the expression of either caStat3β or U-Stat3, as these variants showed the strongest tumor-suppressive or -promoting activities in p19ARF−/− hepatocytes, respectively (Fig. 1). SC injection of p19ARF−/−-positive MMH-R cells expressing caStat3β into mice showed an eight-fold increased tumor formation, compared to MMH-R, whereas the expression of U-Stat3 lowered tumor generation by approximately 1.5-fold (Fig. 3B). After the tail vein injection of cells, MMH-R-caStat3β cells exhibited enhanced lung colonization, whereas MMH-R-U-Stat3 hepatocytes showed lower numbers and a reduced size of lung metastasis (Fig. 3C, D). From these data, we conclude that p19ARF modulates pro- and anti-oncogenic activities of Stat3 during HCC progression.

**Up-Regulation of p19ARF Is Associated with DEN-Induced Tumor Formation in Stat3fl/fl Mice.** Stat3 was recently shown to be required for DEN-induced HCC development. In accord with these data, we observed a reduction in the number and size of liver tumors in Stat3Δh, relative to Stat3fl/fl, mice after DEN treatment (unpublished data). To confirm that the pro-oncogenic role of Stat3 in this background would correlate with p19ARF, we analyzed samples of DEN-induced liver tumors. Indeed, p19ARF was remarkably high during HCC development in Stat3fl/fl mice, whereas DEN-induced liver tumors of Stat3Δh mice showed strongly reduced levels of p19ARF (Fig. 4A). As expected, activation of pY-Stat3 was observed in tumor sections from DEN-treated Stat3fl/fl mice (Fig. 4B). These data show that pY-Stat3 activation is linked to the presence of p19ARF during DEN-induced tumor formation (Fig. 4A), underlining the functional interaction of p19ARF and pY-Stat3 in tumor growth.

**p14ARF Modulates Stat3 Activation During Human HCC Development.** To bridge mouse to human hepatocarcinogenesis, we analyzed an established human HCC cell line for the expression of p14ARF, the human homolog of p19ARF. Real-time PCR analysis showed that human Hep3B cells express p14ARF (Supporting Fig. 3A) and activate Ras/mitogen-activated protein kinase signaling effectors, as described. To investigate the effects of Stat3 in the...
presence or absence of p14ARF, we introduced short-hairpin RNAs (shRNAs) targeted against p14ARF (sh-p14-1 and sh-p14-2) as well as a mixture of both shRNAs (sh-p14-3) into Hep3B cells. Expression of p14ARF was almost eliminated after shRNA expression (Supporting Fig. 3B,C). Hep3B and corresponding shRNA cell lines were SC injected into SCID mice to examine tumorigenesis. Knockdown of p14ARF by the expression of sh-p14-3 was accompanied by a significant down-regulation of pY-Stat3 in vivo, as observed by immunohistochemistry and immunoblotting of tumors (Fig. 5A-C). Notably, tumor volumes of Hep3B cells expressing sh-p14 were comparable to control cells (Fig. 5D). A persistent down-regulation of p14ARF was confirmed after the recultivation of cells from SC tumors (Fig. 5E). From these data, we conclude that the tumor-suppressive function of Stat3 in the absence of p14ARF is circumvented by inhibition of pY-Stat3 in vivo.

p14ARF Acts Downstream of Jak-Mediated Stat3 Phosphorylation. Next, we analyzed whether de novo RNA and protein synthesis would affect pY-Stat3 activation and whether it would depend on the presence of p14ARF. Interestingly, the inhibition of either transcription or translation, using actinomycin D or cycloheximide, respectively, reduced pY-Stat3 levels in both Hep3B, as well as in Hep3B-sh-p14_3, cells, while keeping total Stat3 levels unaffected (Fig. 6A,B). Comparable results were obtained by

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**Fig. 3.** Expression of caStat3 is pro-oncogenic in p19ARF-expressing hepatocytes transformed with oncogenic Ras (MMH-R). (A) Expression of p19ARF in primary hepatocytes (prim. hep.), as well as MMH-D3 and MIM-1-4 hepatocytes, was analyzed by linear semiquantitative RT-PCR. RhoA is shown as the loading control. (B) Tumor formation after SC injection of MMH-R and MMH-R-Stat3 mutants into SCID mice. (C) H&E stainings of lung sections after tail vein injection of cells. (D) Quantification of metastatic colonies according to size. Statistical significance is indicated with asterisks (*P < 0.05, **P < 0.01, ***P < 0.005). Error bars depict SD from at least three individual experiments.

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**Fig. 4.** Up-Regulation of p19ARF in DEN-induced HCC of Stat3fl/fl mice. (A) p19ARF expression analyzed by linear semiquantitative RT-PCR in untreated liver samples, as well as in DEN-induced tumors, of Stat3fl/fl and Stat3Dhc mice. Constitutive expression of RhoA is shown as the loading control. (B) Sections of DEN-induced liver tumors were stained with H&E or with anti-Stat3 or anti–phospho-Stat3 antibodies. Insets show magnification of tumor sections.
employing human PLC/PRF/5 hepatoma cells (data not shown). These data suggest that de novo synthesis of an upstream mediator is essential for pY-Stat3 activation in Hep3B hepatoma cells. However, pY-Stat3 activation occurs in a mode independent of p14ARF expression.

To study the impact of p14ARF on canonical Jak-Stat signaling, Hep3B cells and those showing a knockdown of p14ARF were treated with a pan-Jak inhibitor (blocking Jak1, Jak 2, Jak3, and Tyk2 activity). The pan-Jak inhibitor efficiently blunted pY-Stat3 activation upon interleukin-6 (IL-6) treatment independently of p14ARF expression (Fig. 6C). PLC/PRF/5 hepatoma cells revealed comparable results (data not shown). These findings suggest that Jak activity mainly causes pY-Stat3 activation, irrespective of p14ARF levels, implicating that the control of oncogenic Stat3 function by p14ARF occurs downstream of Stat3 phosphorylation.

**Discussion**

This study shows that Stat3 is able to execute both pro- and anti-oncogenic functions, depending on p19ARF/p14ARF expression, during Ras-mediated HCC development. CaStat3 acts in a tumor-suppressive manner in Ras-transformed p19ARF−/− hepatocytes as well as in a tumor-promoting manner in hepatocytes expressing p19ARF. Strikingly, the Y705F mutant (U-Stat3) showed the opposite effect. In line with these findings, tumors derived from the human HCC cell line Hep3B show reduced pY-Stat3 upon p14ARF silencing. In this scenario, human HCC cells counteract the tumor-suppressive effects of Stat3, as observed
in the murine p19ARF⁻/⁻ model, and prevent diminished tumor growth. p14ARF levels in HCC cells affect pY-Stat3 activation in vivo, whereas pY-Stat3 activation mainly induced by Jak seems to be independent of p14ARF expression in vitro.

p14ARF is a potential target for inactivation in HCC due to its positive role in p53 stabilization by promoting MDM2 degradation. In accord with our results, the p14ARF-negative HCC cell lines, HepG2 and PLC/PRF/5, are less sensitive to Stat3 inhibitor NSC 74859 treatment, whereas p14ARF expressing HuH-7 and SNU-398 cells show reduced cell proliferation after the administration of NSC 74859. Our observations in murine hepatocytes suggest that a tumor-suppressive Stat3 function depends on p19ARF deficiency, but might be independent of both p16INK4B and p53 inactivation, because p19ARF⁻/⁻ hepatocytes express p16INK4A and show p53 response. In line with this, the human hepatoma Hep3B cells used in this study harbored a p53 mutation that did not affect the response of reduced pY-Stat3 activation in p14ARF knocked-down cells. Furthermore, pro-oncogenic Stat3 functions were observed in hepatitis B virus–positive HCC cell lines, such as SNU-182 or SNU-387, which express mutated and inactivated p53, indicating tumor-promoting Stat3 functions independent of p53.

Modulation of pro- or anti-oncogenic Stat3 functions through tumor suppressors has been described in different cancers. In glioblastoma, deficiency in PTEN induces a malignant transformation of astrocytes upon Stat3 knockout, arguing for anti-oncogenic functions of Stat3. Recently, we also described a dual role of Stat3 in ApcMin/+ mice, where Stat3 promotes early microadenoma formation, whereas Stat3 deficiency in intestinal epithelial cells increased later stage carcinoma progression, associating with nuclear β-catennin and impaired Ceacam1 expression. In addition, we showed that caStat3 blocked c-myc-induced transformation of p53⁻/⁻ mouse fibroblasts. These findings indicate that Stat3 functions are modulated by various tumor suppressors.

Despite the inability of oncogenic Ras to drive Stat3 tyrosine phosphorylation or nuclear translocation, Ras transformation was found to be impaired in the absence of Stat3. Similar results were obtained upon mouse mammary tumor progression, showing that Stat3 is indispensable for the metastasis of ErbB2-activated cancer cells to the lung. In contrast, our data suggest that Stat3 is not required for Ras transformation of hepatocytes in the absence of p19ARF (Fig. 2). Furthermore, Stat3 regulates metabolic functions in mitochondria, requiring Ser727 phosphorylation that supports Ras-dependent malignant transformation. Our data exclude an involvement of Ser727 phospho-Stat3 in the dual role of Stat3 in Ras-transformed hepatocytes, as expression of the C-terminally truncated Stat3β either suppressed or promoted tumor growth dependent on p19ARF expression similarly to gain-of-function studies using full-length Stat3α (Figs. 1 and 3B-D).
U-Stat3, harboring the Y705F mutation, abrogated tumor suppression and even enhanced tumor formation (Fig. 1), probably driven by the expression of a gene set specific for U-Stat3 and its putative interaction partners. Because p14ARF/p19ARF is known to interact with a multitude of proteins from different functional classes, it is conceivable that a putative factor, designated ARF-X, is involved in the U-Stat3-driven transcriptional control, as hypothesized in Fig. 7. The occupation of ARF-X by p14ARF/p19ARF could be responsible for the different outcome in the presence of p14ARF/p19ARF (Fig. 1 versus Fig. 3B-D). Jak activity, which might be crucially involved in pY-Stat3 activation of HCC cells, is not altered by p14ARF in human HCC cells in vitro (Fig. 6). However, pY-Stat3 is affected in tumors generated by p14ARF knocked-down Hep3B cells to overcome tumor-suppressive actions (Fig. 5A-C). Presumably, the in vivo environment, including tumor-stroma interactions, allows the tumor to act in a manner distinctive from malignant cells in vitro. In this scenario, the identification of ARF-X is to be the focus of future experiments with highest priority.

Activation of Stat3, occurring in the majority of HCC patients, suggests a critical role in liver cancer. Stat3 is considered a potential target for therapeutic intervention, as Stat3 inhibition represses experimental tumors, but shows little side effects. We provide the first evidence that p14ARF determines whether Stat3 acts with pro- or anti-oncogenic function in HCC cells. The link of Stat3 with p14ARF might be of prognostic value for HCC therapy. Treatment of pY-Stat3-positive HCC patients showing loss of p14ARF with Stat3 inhibitors could have adverse effects on cancer progression, thus opening new aspects for individualized medicine.

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References
1. Levy DE, Lee CK. What does Stat3 do? J Clin Invest 2002;109:1143-1148.
2. Yu H, Jove R. The STATs of cancer—new molecular targets come of age. Nat Rev Cancer 2004;4:97-105.
3. Bromberg JF, Wrzeszczynska MH, Devgan G, Zhao Y, Pestell RG, Albanese C, et al. Stat3 as an oncogene. Cell 1999;98:295-303.
4. Dewilde S, Verceil A, Chiarle R, Poli V. Of alphas and betas: distinct and overlapping functions of Stat3 isoforms. Front Biosci 2008;13:6501-6514.
5. Levy DE, Inghirami G. STAT3: a multifaceted oncogene. Proc Natl Acad Sci U S A 2006;103:10151-10152.
6. Yu H, Korylewski M, Pardoll D. Crosstalk between cancer and immune cells: role of Stat3 in the tumour microenvironment. Nat Rev Immunol 2007;7:41-51.
7. de la Iglesia N, Konopka G, Puram SV, Chan JA, Bachoo RM, You MJ, et al. Identification of a PTEN-regulated Stat3 brain tumor suppressor pathway. Genes Dev 2008;22:449-462.
8. Ecker A, Simma O, Hoell B, Kenner L, Beug H, Moriggl R, et al. The dark and the bright side of Stat3: proto-oncogene and tumor-suppressor. Front Biosci 2009;14:2944-2958.
9. Musteanu M, Blas L, Mair M, Schleder M, Bilban M, Tauber S, et al. Stat3 is a negative regulator of intestinal tumor progression in Apc(Min) mice. Gastroenterology 2010;138:1003-1011.
10. Taub R. Liver regeneration: from myth to mechanism. Nat Rev Mol Cell Biol 2004;5:836-847.

11. He G, Karin M. NF-kappaB and STAT3: key players in liver inflammation and cancer. Cell Res 2011;21:159-168.

12. Zhang CH, Yu GL, Jia WD, Li JS, Ma JL, Ren WH, et al. Activation of STAT3 signal pathway correlates with twist and E-cadherin expression in hepatocellular carcinoma and their clinical significance. J Surg Res 2010; doi:10.1016/j.jss.2010.10.030.

13. Li WC, Ye SL, Sun RX, Liu YK, Tang ZY, Kim Y, et al. Inhibition of growth and metastasis of human hepatocellular carcinoma by anti-sense oligonucleotide targeting signal transducer and activator of transcription 3. Clin Cancer Res 2006;12:7140-7148.

14. Yang SF, Wang SN, Wu CF, Yeh YT, Chai CY, Chunag SC, et al. Altered p-STAT3 (tyr705) expression is associated with histological grading and intratumour microvesel density in hepatocellular carcinoma. J Clin Pathol 2007;60:642-648.

15. Lau CK, Yang ZF, Lam SP, Lam CT, Ngai P, Tam KH, et al. Inhibition of Stat3 activity by YC-1 enhances chemo-sensitivity in hepatocellular carcinoma. Cancer Biol Ther 2007;6:1900-1907.

16. Lin L, Amin R, Gallicano GI, Glasgow E, Jogunoori W, Jessup JM, et al. Inhibition of RhoA activity in hepatocellular carcinoma by antisense oligonucleotide targeting signal transducer and activator of transcription 3. J Hepatol 2010;52:921-929.

17. Valvisi DF, Ladu S, Conner EA, Seo D, Hsieh JT, Factor VM, et al. Inactivation of Ras GTPase-activating proteins promotes unrestrained activity of wild-type Ras in human liver cancer. J Hepatol 2011;54:311-319.

18. Pollice A, Vivo M, La Mantia G. The promiscuity of ARF interactions with disrupted TGF-beta signaling. Oncogene 2009;28:961-972.

19. He G, Yu GY, Temkin V, Ogata H, Kuntzen C, Sakurai T, et al. Hepatocyte IKKbeta/NF-kappaB inhibits tumor promotion and progression by preventing oxidative stress-driven STAT3 activation. Cancer Cell 2010;17:286-297.

20. Yang JB, Stark GR. Roles of unphosphorylated STATs in signaling. Cell 2008;134:443-451.

21. Ender L, Villanueva A, Tovar V, Sia D, Chiang DY, Llovet JM. Inhibition of Stat3 activity by YC-1 enhances chemo-sensitivity in hepatocellular carcinoma. Cancer Biol Ther 2007;6:1900-1907.

22. He G, Karin M. NF-kappaB and STAT3: key players in liver inflammation and cancer. Cell Res 2011;21:159-168.

23. Zender L, Villanueva A, Tovar V, Sia D, Chiang DY, Llovet JM. Inhibition of Stat3 activity by YC-1 enhances chemo-sensitivity in hepatocellular carcinoma. Cancer Biol Ther 2007;6:1900-1907.

24. Paulitschke V, Ye SL, Sun RX, Liu YK, Tang ZY, Kim Y, et al. Inhibition of growth and metastasis of human hepatocellular carcinoma by anti-sense oligonucleotide targeting signal transducer and activator of transcription 3. Clin Cancer Res 2006;12:7140-7148.

25. Gotzmann J, Huber H, Thallinger C, Wolschek M, Jansen B, Schulte-Hermann R, et al. Activation of STAT3 signal pathway correlates with twist and E-cadherin expression in hepatocellular carcinoma and their clinical significance. J Surg Res 2010; doi:10.1016/j.jss.2010.10.030.

26. Miura M, Fuchs E, Huber H, Beug H, Schulte-Hermann R, Mikulits W. Immortalized p19ARF null hepatocytes restore liver injury and generate hepatic progenitors after transplantation. Hepatology 2004;39:628-634.