Celecoxib Inhibits Interleukin-6/Interleukin-6 Receptor-Induced JAK2/STAT3 Phosphorylation in Human Hepatocellular Carcinoma Cells

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Abstract

Growing evidence demonstrates an association between chronic liver inflammation and hepatocellular carcinoma (HCC) development. Signal transducer and activator of transcription 3 (STAT3), which is associated with inflammation and cellular transformation, is constitutively activated in human HCC tissues, but not in normal human liver tissues. Although interleukin-6 (IL-6) is elevated in the serum of patients with HCC, it is not fully understood whether STAT3 constitutive activation is positively correlated with autocrine IL-6 secreted by HCC cells. Here we reported that in HCC cells the elevated levels of both IL-6 and IL-6 receptor (IL-6R, gp80), not IL-6 alone, correlated with STAT3 activation. We also explored whether the anti-cancer effects of Celecoxib, an anti-inflammatory drug, may be due to the inhibition of the IL-6/STAT3 pathway in HCC cells. Our results demonstrated that Celecoxib decreased STAT3 phosphorylation through reducing JAK2 phosphorylation and caused apoptosis in HCC cells. Celecoxib could also block exogenous IL-6-induced STAT3 phosphorylation and nuclear translocation. Moreover, we observed more significant inhibition of cell viability when Celecoxib was combined with Doxorubicin or Sorafenib. We conclude that the elevated levels of IL-6/IL-6R may be correlated with STAT3 activation in HCC cells. Celecoxib may be a candidate for HCC therapy through blocking IL-6/STAT3 pathway and can be combined with other anti-cancer drugs to reduce drug resistance caused by IL-6/STAT3 signals.
Introduction

Primary liver cancer is the sixth most common cancer of all human cancers worldwide and is a major health problem due to the poor prognosis (1). Hepatocellular carcinoma (HCC) accounts for more than 85% of all primary liver cancers, with a five-year survival rate of 9% and a median survival time of less than one year (2-4). Epidemiological studies have established that HCC occurs in association with hepatitis B virus and ethanol, but the molecular mechanisms have not been fully known. Over the past few years, growing evidence has demonstrated that cytokines, like IL-6, and transcription factors, such as NF-κB and STAT3, play an important role in HCC development (2, 5-9). Patients with HCC show elevated levels of IL-6 in their serum as compared to those with liver cirrhosis or to healthy individuals. In addition, the level of IL-6 is remarkably high in HCC stage III patients (5). Signal transducer and activator of transcription 3 (STAT3), a major transducer to mediate the signal from IL-6 to the nucleus, is found significantly correlated with the prognosis of HCC patients (9), indicating that IL-6/STAT3 signaling pathway might be a therapeutic target.

 Constitutively activated STAT3 may induce tumor formation in nude mice and is frequently detected in various human cancers (10-12). STAT3 may be involved in oncogenesis, cell proliferation, angiogenesis, immune evasion, and apoptotic resistance (13-15). Growing evidence has demonstrated that a blockade of constitutively activated STAT3 can cause apoptosis in vitro, inhibit tumor growth in vivo, and enhance the sensitivity to chemotherapy and radiotherapy (11, 16-21).

IL-6 induces STAT3 phosphorylation at tyrosine residue 705 through IL-6 receptors (IL-6R and gp130) and Janus family kinases (JAK) (22, 23). Phosphorylated STAT3 molecules dimerize and
translocate from the cytoplasm to the nucleus, where they bind to specific DNA elements to regulate the downstream genes, including Bcl-XL, CyclinD1, HIF-1α, Mcl-1, p53, and VEGF. These genes are involved in cell proliferation, angiogenesis, and apoptosis (24, 25).

In this study, we reported that the levels of IL-6/IL-6R, not IL-6 alone, correlated with the activation of STAT3 in HCC cells. In addition, Celecoxib, a COX-2 inhibitor, blocked IL-6/IL-6R-induced STAT3 phosphorylation in HCC cells through suppressing JAK2 phosphorylation and caused cell death. Although many STAT3 small molecule inhibitors have been described, none of them is currently selected for clinical trials. Celecoxib may be a candidate for HCC therapy or may be combined with other anti-cancer drugs.

**Materials and Methods**

**Cell Culture**

HCC cell lines, Hep3B, HepG2, Huh-7, SNU-387, and SNU-449 were obtained from American Type Culture Collection (ATCC, Manassas, VA). The cell lines have been characterized by ATCC and we have not done any further characterization. Hep3B (HBV positive) and HepG2 (HBV negative) cells were cultured in Minimum Essential Medium, Eagle (MEM) (ATCC) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. Huh-7 (HBV negative) cells were cultured in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin. SNU-449 (HBV positive), SNU-387 (HBV positive) and SNU-398 (HBV positive) cells were cultured in RPMI 1640 medium (ATCC) supplemented with 10% FBS and 1% penicillin/streptomycin. Human hepatocytes (HH) were purchased from ScienCell Research Laboratories (Carlsbad, CA) and were cultured in Hepatocyte Medium (HM,
ScienCell) supplemented with 5% FBS and 1% penicillin/streptomycin. Interleukin-6 was purchased from Cell Sciences (Canton, MA).

**Western Blot**

Cells were washed by ice cold phosphate-buffered saline (PBS) buffer and lysed by ice cold RIPA buffer containing proteasome inhibitor cocktail and phosphatase inhibitor cocktail. The lysates were then spun at 13,200 rpm for 10 minutes at 4°C and the supernatant was collected. Protein samples were separated by SDS-PAGE, transferred onto PVDF member and immunoblotted with appropriate antibody. Antibodies to pSTAT3<sup>Y705</sup>, pSTAT3<sup>S727</sup>, STAT3, cleaved caspase-3, pJAK2<sup>Y1007/1008</sup>, JAK2, pJAK1<sup>Y1022/1023</sup>, JAK1, GAPDH and HRP conjugated secondary antibody were from Cell Signaling Technology (Beverly, MA). The target protein was examined by chemiluminescence (Cell Signaling Technology).

**MTT Assay**

Cells were seeded in a 96-well plate and were treated as indicated for 24 hours. After the treatment, 25 µl of 3-(4,5-dimethylthiazolyl)-2,5-diphenyltetrazolium bromide (MTT, Sigma, St. Louis, MO) was added to each well. After 3-hour incubation at 37°C, 100 µl of N, N-dimethylformamide (Sigma) solubilization solution was added to each well. Absorbance was read at 595 nm.

**Immunohistochemistry**

Liver tumor tissue assay was purchased from BioChain Institute, Inc (Hayward, CA). Immunohistochemical labeling was performed according to the manual of the tissue assay. Sections were labeled using primary antibodies against pSTAT3<sup>Y705</sup> (Cell Signaling) and IL-6
(ab6672, Aabcam Inc, Cambridge, MA). The staining was performed with Histostain-Plus Kit (Invitrogen, Camarillo, CA). The frequency of IL-6 and pSTAT3\(^{Y705}\) positive was analyzed as described by Lin et al. (26).

**Immunofluorescence**

Cells were seeded on a glass slide and were treated as indicated. After the treatment, the cells were washed with cold PBS buffer and were fixed with cold methanol for 15 minutes at -20°C. After two quick washing with cold PBS, the slide was blocked with 5% normal goat serum and 0.3% Triton X-100 in PBS for one hour at room temperature. Then the slide was incubated with STAT3 antibody (Cell Signaling) at 4°C. After overnight incubation, the slide was washed with PBS-T solution (PBS plus 0.1% Tween-20) and then was incubated with Alexa Fluor 594 secondary antibody (Molecule Probe, Invitrogen) for one hour at room temperature. The cells were mounted with Vectashield HardSet mounting medium with DAPI (Vector Laboratories, Burlingame, CA). Pictures were captured by Leica Microsystems (Bannockburn, IL).

**STAT3 DNA Binding Assay**

STAT3 DNA binding assay was performed according to the manual of STAT3 DNA binding ELISA Kit (Active Motif, Carlsbad, CA). Briefly, cells were seeded in a 10 cm plate. After the treatment, nuclear protein was extracted and was mixed with STAT3 DNA probe. The protein/DNA complex was then transferred into an ELISA assay plate. After the incubation with primary and secondary antibodies, the developing solution was added. The absorbance was read at 450 nm.

**Reverse Transcription-Polymerase Chain Reaction**
RNA was extracted using RNeasy kit (Qiagen, Valencia, CA). Reverse transcription was performed using Omniscript reverse transcription kit (Qiagen). PCR conditions were: 94°C 5 minutes followed by 30 cycles of 94°C 30 seconds, 55°C 30 seconds and 72°C 1 minute with a final extension of 72°C 10 minutes.

**Apoptosis Assay**

Apoptosis was measured with caspase3/7 assay (Promega, Madison, WI) according to the manufacture’s protocol. Briefly, cells were seeded into a 96-well plate. After the treatment, 100 µl of Apo-One Caspase3/7 reagent was added to each well and was incubated at 37°C for 30 minutes. The fluorescence was measured at an excitation wavelength range of 485 nm and an emission wavelength range of 530 nm.

**Colony formation**

Cells were treated for specific time point as indicated. Then the treated cells were trypsinized, stained with trypan blue, and counted. The same number of cells were then seeded in 10 cm plates and allowed to grow. After 3 weeks, the cells were fixed by cold methanol and stained with 1% crystal violet in 25% methanol.

**Statistical analysis**

Data were presented as the mean ± SEM in which the mean values were compared. Statistical analyses were conducted by Student’s t test. P value < 0.05 was considered statistically significant and was indicated with *.

**Results**
STAT3 activation is positively correlated with the levels of IL-6/IL-6R in HCC cells. To investigate whether STAT3 activation would positively correlate with the levels of IL-6 secreted by HCC cells, we first analyzed the levels of phosphorylated STAT3 in six HCC cell lines and in primary human hepatocytes (HH). HepG2, Huh-7, SNU-387, and SNU-449 cells showed an increase in phosphorylated STAT3 as compared to HH cells (Figure 1A). To explore whether these four cell lines with higher levels of phosphorylated STAT3 may have higher levels of IL-6, we examined the levels of IL-6 in these cells. Since most IL-6 protein was secreted into the medium, IL-6 protein in these cells was not detectable by western blot. Therefore, we evaluated IL-6 mRNA in these cells by RT-PCR. The result was beyond our expectations. SNU-387 and SNU-449 cells had higher levels of IL-6 than HH cells (Figure 1B), whereas HepG2 and Huh-7 cells had higher levels of phosphorylated STAT3, but lower levels of IL-6. To explore whether neutralizing IL-6 would decrease STAT3 phosphorylation, we treated Hep3B, SNU-449, and SNU-387 cells with 500 ng/ml of anti-IL-6 antibody for 72 hours. The treatment decreased the levels of phosphorylated STAT3 in SNU-449 and SNU-387 cells and induced caspase3 activation, whereas the same treatment did not increase caspase3 activation in Hep3B cells (Figure S1).

To confirm these results, immunohistochemistry for IL-6 and pSTAT3 was also performed on normal liver tissues (3 cases), as well as on tissues from patients with viral subfulminant hepatitis (2 cases) or hepatocellular carcinoma (HCC, 25 cases). Two normal tissues were moderate (+++) IL-6 immunostaining and one was weak (+). One intense (++++) IL-6 and one moderate (++) IL-6 were observed on two viral subfulminant hepatitis samples. Seven of twenty-five HCC samples were intense (+++), and the other sixteen HCC samples were moderate (11 cases) and weak (7 cases). As for pSTAT3, all of the three normal samples were negative. One viral subfulminant
hepatitis sample was negative, and one was weak. Three HCC samples were intense, eight samples were moderate, eight samples were weak, and seven samples were negative (Figure 1C). Overall, both benign and malignant tissues were IL-6 positive compared with their connective tissues, such as blood vessels, whereas 20% of benign samples and 76% of HCC samples were pSTAT3705 positive. Given the results from cell lines and tissues, these data suggest that IL-6 is a factor for STAT3 activation in HCC tissues and cancer cell lines, but not in normal cells and benign tissues. Even in HCC samples, other risk factors rather than IL-6 may exist to activate STAT3 in HCC cells.

Since IL-6 receptors including both IL-6R (gp80) and gp130 can also mediate STAT3 activation during tumorigenesis (22, 27, 28), we analyzed the mRNA of IL-6R and gp130 in HCC cells by RT-PCR. The levels of IL-6R were higher in all six cancer cell lines than in HH cells. Additionally, IL-6R was remarkably overexpressed in HepG2, Huh-7, and SNU-449 cells (Figure 1D). The results of IL-6R were quantified by GAPDH using Image J software. However, no significant overexpression of gp130 was observed in HCC cell lines (Figure 1D). Based on the results of IL-6 and IL-6R (Figure 1A, 1B and 1D), our data suggest a trend toward a positive correlation between the levels of pSTAT3705 and the levels of IL-6/IL-6R, but not those of IL-6 alone, in HCC cells.

**Celecoxib inhibits IL-6/IL-6R-induced STAT3 phosphorylation in HCC cells.** Some anti-inflammatory drugs have shown inhibitory effects on tumor growth *in vitro* and *in vivo*, such as Celecoxib, a COX-2 inhibitor. To examine whether the antitumor effects may be due to the inhibition of some inflammatory pathways, such as IL-6/STAT3 pathway, we examined Celecoxib. Four HCC cell lines with higher IL-6/IL-6R and phosphorylated STAT3 were treated
with Celecoxib for 24 hours. We observed that Celecoxib effectively reduced the levels of pSTAT3Y705, whereas it had no effects on pSTAT3S727 and total STAT3 (Figure 2A). To explore the mechanism of its inhibitory effects on pSTAT3Y705, JAK1 and JAK2 were evaluated. pJAK2Y1007/1008 was suppressed in all four HCC cell lines with the treatment of Celecoxib, whereas pJAK1Y1022/1023 was not detectable. Neither JAK1 nor JAK2 changed upon Celecoxib treatment (Figure 2A).

We next examined the effects of Celecoxib on STAT3 DNA binding ability using Huh-7 and SNU-449 cell lines. As illustrated in Figure 2B, Celecoxib reduced STAT3 DNA binding ability in a dose-dependent manner. To further investigate whether Celecoxib would affect STAT3 downstream genes, we used RT-PCR to examine the mRNA expression of Bcl-2, Bcl-XL, CyclinD1, HIF-1α, Survivin, and VEGF. As shown in Figure 2C, the treatment of Celecoxib down-regulated the mRNA expression of these genes.

**Celecoxib induces apoptosis in HCC cells and reduces cell viability.** To investigate whether blocking pSTAT3Y705 with Celecoxib would cause apoptosis, we treated HCC cells (Huh-7, HepG2, SNU-387, and SNU-449) with different concentrations of Celecoxib and analyzed the levels of cleaved caspase-3 and the activity of caspase3/7. The results demonstrated that both the levels of cleaved caspase-3 and the activity of caspase-3/7 were enhanced by Celecoxib treatment (Figure 3A and Figure 3B). In addition, the treatment significantly decreased cell viability of all four cell lines (Figure 3C), which was consistent with the apoptotic results. Human primary hepatocytes and HCC cells (Hep3B and SNU-398) with lower levels of phosphorylated STAT3 were less sensitive to the treatment of Celecoxib (Figure S2). Moreover,
neutralization of IL-6 in SNU-449 and SNU-387 cells with anti-IL-6 antibody desensitized the cells to further Celecoxib-induced apoptosis (Figure S3).

Clonogenic assay was also performed to investigate the colony forming ability after Celecoxib treatment (Figure 3D). Our results showed that the colony forming ability were significantly reduced by Celecoxib treatment in a dose-dependent fashion.

**Celecoxib inhibits exogenous IL-6-induced pSTAT3^{Y705}.** To investigate whether Celecoxib may block exogenous IL-6-induced STAT3 activation, Hep3B cells with low levels of IL-6 and pSTAT3^{Y705} were pre-treated with 25 μM and 50 μM of Celecoxib for 2 hours, followed by 25 ng/ml of IL-6 for 30 minutes. After the treatment, pSTAT3^{Y705} and STAT3 were analyzed by western blot. The pre-treatment with 25 μM of Celecoxib inhibited IL-6-induced pSTAT3^{Y705}, and the pre-treatment with 50 μM of Celecoxib effectively blocked pSTAT3^{Y705} (Figure 4A). To further explore whether Celecoxib inhibited IL-6-induced pSTAT3^{Y705} through repressing JAK2 phosphorylation, we examined the effect of Celecoxib on the phosphorylation of JAK2 caused by IL-6 stimulation. Figure 4B clearly showed that IL-6 induced JAK2 phosphorylation, whereas Celecoxib pre-treatment prevented its phosphorylation. To confirm whether IL-6 would induce phosphorylated STAT3 nuclear accumulation and total STAT3 nuclear translocation and whether Celecoxib pre-treatment would block this process, Hep3B cells were pre-treated with 50 μM of Celecoxib for 2 hours, followed by 25 ng/ml of IL-6 for 30 minutes. Phosphorylated STAT3 accumulated in the nucleus in response to IL-6 treatment, whereas Celecoxib pre-treatment blocked this process (Figure 5A). STAT3 was in the cytoplasm in the absence of IL-6. Following IL-6 treatment, STAT3 translocated to the nucleus, whereas Celecoxib pre-treatment blocked this process (Figure 5B).
Inhibition of STAT3 by Celecoxib reduces cell viability more significantly when combined with other anti-cancer drugs. We previously reported that the blockade of IL-6/STAT3 pathway is able to overcome IL-6-induced drug resistance in HCC cells (21). We also showed in Figure 2B that Celecoxib could block the IL-6/STAT3 pathway. To investigate whether inhibition of STAT3 by Celecoxib would lead to more cell death when combined with other anti-cancer drugs, we treated SNU-449 cells with Doxorubicin (Figure 6A) or Sorafenib (Figure 6B) with or without Celecoxib. The treatment of Doxorubicin or Sorafenib reduced viable cell numbers more significantly in the presence of Celecoxib (Figure 6A and 6B). Additionally, cells treated with Celecoxib and Sorafenib significantly lost colony forming ability as compared to the effect of either agent (Figure 6C).

Discussion

STAT3 is activated in response to infection, hormones, growth factors, and cytokines, as well as by the activation of intracellular kinases (16). Constitutive STAT3 activation plays a critical role in tumor formation and development in a variety of primary human cancers and cell lines. Cellular transformation by various factors, such as virus infection or protein tyrosine kinase activation, accompanies aberrant activation of STAT3 (29, 30), which may promote tumor cell proliferation through the upregulation of CyclinD1, Myc, and Pim-1 (30-33) and may suppress apoptosis through the upregulation of Bcl-XL and Survivin (34, 35). It may also mediate invasion through the upregulation of MMP1 and MMP2 (36, 37) and increase angiogenesis and metastasis through the upregulation of VEGF and Twist (13, 38). In addition, activated STAT3 may suppress anti-tumor immunity through the inhibition of Th1 immuno-stimulatory molecules.
Downregulation of constitutively activated STAT3 not only induces apoptosis in cancer cells but also overcomes chemoresistance and radioresistance (30).

Since no gain-of-function STAT3 mutants have been found, it seems important to investigate the factors for STAT3 activation in different types of human cancer. IL-6 is involved in STAT3 activation in a variety of human cancers. In the liver, short term treatment with IL-6 provides hepatoprotection against various liver injuries by stimulating liver regeneration (40-43). However, a murine model of HCC showed that when mice were treated with diethylnitrosamine (DEN), which can induce hepatocellular carcinogenesis, males were more likely to develop HCC than females. The gender disparity was due to the higher levels of IL-6 in males and was not present in IL-6 knockout mice, indicating an association between IL-6 and HCC progression (44). Another mouse study demonstrates that inflammatory cytokines, including IL-6, promote liver cancer metastasis (45).

Although the serum levels of IL-6 are elevated in patients with HCC, and STAT3 is constitutively activated in HCC cells (5, 26), no direct evidence shows that IL-6 secreted by HCC cells is correlated with STAT3 constitutive activation. In this study, we demonstrated that IL-6 was a critical factor that contributed to STAT3 constitutive activation in HCC cells. Some HCC cells and tissues had higher pSTAT3Y705 and higher levels of IL-6. However, some HCC cells had higher pSTAT3Y705 but lower levels of IL-6, and some had lower pSTAT3Y705 but higher levels of IL-6. Moreover, IL-6 was also detected in normal human liver cells and tissues, but their pSTAT3Y705 was not detectable or was very weak. These results made us hypothesize that IL-6 receptors IL-6R or gp130 may be another factor for STAT3 constitutive activation in HCC because IL-6R has been found overexpressed in human ovarian cancer cells, and the
elevated IL-6R activates STAT3 in these cancer cells. (28). gp130 can also activate STAT3 during colitis-associated tumorigenesis (22). Our results demonstrated that it was IL-6R, but not gp130, that was overexpressed and activated STAT3 in some HCC cells. gp130 was not found overexpressed either in ovarian cancer cells. Taken together, the elevated levels of IL-6/IL-6R, not IL-6 alone, led to STAT3 constitutive activation in HCC cells.

In the second part, we hypothesized that whether the anti-cancer effects of some anti-inflammatory drugs may be due to the blockage of some inflammatory pathways, like IL-6/STAT3. Celecoxib, a COX-2 inhibitor, has shown anti-cancer effects in colon carcinoma, lung carcinoma, and prostate cancer in vitro and in vivo through different mechanisms, such as blocking pAKT or pERK (46-48). Here we showed that Celecoxib could inhibit IL-6/IL-6R-induced STAT3 activation in HCC cells through blocking JAK2 phosphorylation. Moreover, the anti-inflammatory effects of Celecoxib may be also due to the blockage of JAK2/STAT3 because COX-2 is upregulated by STAT3 (25). Finally, we showed that Celecoxib could be combined with other anti-cancer agents to overcome the drug resistance. IL-6/STAT3 targets some genes involved in anti-apoptosis, such as Bcl-2, Bcl-xL, and Survivin. They are thought to be involved in resistance to conventional cancer treatment. Celecoxib treatment decreased the expression of these STAT3 downstream genes, which inhibited the resistance of cancer cells to anti-cancer drugs. Therefore, when other anti-cancer drugs were combined with Celecoxib, the treatment caused more cell death. In summary, our results suggest that Celecoxib may be a candidate for HCC therapy through inhibiting IL-6/STAT3 pathway and may be combined with other anti-cancer drugs to reduce drug resistance caused by STAT3.

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**Figure Legends**

Figure 1 STAT3 activation is positively correlated with IL-6/IL-6R in HCC cells. (A) The levels of pSTAT3\textsuperscript{Y705} and STAT3 in HCC cell lines and normal liver cells were analyzed by western blot. (B) The levels of IL-6 mRNA were analyzed by RT-PCR. (C) The IL-6 and pSTAT3\textsuperscript{Y705} status in liver tissues was evaluated by immunohistochemistry. (D) The levels of IL-6R and gp130 in HCC cells and normal liver cells were examined by RT-PCR. The results of IL-6R were quantified by GAPDH using Image J software.

Figure 2 Celecoxib inhibits STAT3 activation. (A) HepG2, Huh-7, SNU-387, and SNU-449 cells were treated with different concentrations of Celecoxib. pSTAT3\textsuperscript{Y705}, pSTAT3\textsuperscript{S727}, STAT3, pJAK2\textsuperscript{Y1007/1008}, JAK1, JAK2, and GAPDH were analyzed by western blot. (B and C) Huh-7 and SNU-449 cells were treated with different concentrations of Celecoxib overnight. STAT3 DNA binding ability and the mRNA expression of STAT3 downstream genes were examined by STAT3 DNA binding assay (B) and RT-PCR (C), respectively. The data from (B) represented three independent results.

Figure 3 Celecoxib induces apoptosis and reduces cell viability. (A and B) HCC cell lines were treated with Celecoxib. The levels of cleaved caspase-3 (A) and the activity of caspase-3/7 were analyzed (B). (C) Cell viability was measured in Celecoxib treated HCC cell lines by MTT assay. (D) SNU-449 cells were treated with Celecoxib. After the overnight treatment, the same
number of living cells with either DMSO treatment or Celecoxib treatment was re-seeded. The cells were allowed to grow in fresh medium without Celecoxib for two weeks. After two weeks, the cells were fixed and stained. The data from (B, C, and D) represented three independent results.

Figure 4 Celecoxib inhibits exogenous IL-6-induced pSTAT3Y705. Hep3B cells were pre-treated with Celecoxib for two hours, followed by 25 ng/ml of IL-6 for 30 minutes. pSTAT3Y705 (A) and pJAK2Y1007/1008 (B) were analyzed by western blot.

Figure 5 Celecoxib blocks IL-6-induced STAT3 nuclear translocation. Hep3B cells were pre-treated with Celecoxib for two hours, followed by 25 ng/ml of IL-6 for 30 minutes. (C) The cellular localization of pSTAT3Y705 (A) and STAT3 (B) was examined by immunofluorescence. Nuclei were stained with DAPI.

Figure 6 Celecoxib induces more cell death when combined with other anti-cancer drugs. (A and B) SNU-449 cells were treated with different concentrations of Doxorubicin (A) and Sorafenib (B) with and without Celecoxib. After 24 hours, cell viability was measured by MTT assay. The data represented three independent results. (C) SNU-449 cells were treated as indicated in Figure 6A. After the treatment, living cells were counted and the same number of cells was re-seeded and cultured for three weeks and four weeks. Colonies were fixed by cold methanol and stained by 1% crystal violet.

References


Figure 2
Figure 5

A

Calvesk (2 h) 25 ng/ml 50 μg/ml
gEF1α

DAPI

B

Calvesk (2 h) 25 ng/ml 50 μg/ml
IL-6 (30 min)

STAT3

Merger
Cancer Prevention Research

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