IL-6 is involved in malignancy and doxorubicin sensitivity of renal carcinoma cells

Yanqiang Chen, Jianzhen Li, Pei Lv, Jiangyan Gao, Mingzheng Wang, and Yongjun Wang

Department of Neurology, Hebei Chest Hospital, Shijiazhuang, Hebei, China; Department of Urology, Hebei Chest Hospital, Shijiazhuang, Hebei, China; Department of Nephrology, Hebei Chest Hospital, Shijiazhuang, Hebei, China; Cardiovascular Department, Hebei Chest Hospital, Shijiazhuang, Hebei, China; Department of Thoracic Surgery, Hebei Chest Hospital, Shijiazhuang, Hebei, China

ABSTRACT
Various survival factors such as the pleiotropic cytokine interleukin-6 (IL-6), a major mediator of inflammation and activator of signal transducer and activator of transcription 3 (STAT3), serve to block apoptosis in cancer cells. Our present study revealed that the expression of IL-6, while not other IL-2, IL-4, IL-8, or IL-10, was significantly elevated in resistance of renal carcinoma cells (RCC) when compared with human renal proximal tubule epithelial cell line HK-2. The inhibition of IL-6 by siRNA can suppress the proliferation, migration and invasion of RCC cells and increase the doxorubicin (Dox) sensitivity. While recombination IL-6 can attenuate the inhibition effects of Dox on proliferation of RCC cells. Further studies indicated that inhibition of IL-6 by siRNA can decrease the phosphorylation of STAT3 in RCC cells. Over expression of STAT3 increased the proliferation, migration and invasion of RCC cells and reversed si-IL-6 induced increase of Dox sensitivity of ACHN and A498 cells. In addition, IL-6 treatment can activate ERK1/2 via increasing its phosphorylation. PD98059, the ERK1/2 inhibitor, attenuated IL-6 induced proliferation and synergistically increased the Dox sensitivity of si-IL-6 transfected ACHN cells. Collectively, our data suggested that IL-6 plays an important role in malignancy and Dox sensitivity of RCC. The targeted inhibition of IL-6 signals might be a promising therapeutic strategy for the treatment of renal cancer.

Introduction
Renal cell carcinoma (RCC) has been reported to account for 2% of all cancers and approximately 90% of renal cancer patients. Clear-cell renal cell carcinoma (ccRCC) is the most common histological subtypes of RCC and accounted for more than 80% cases. Although there were great achievements for early imaging detection and curative resection of the tumor within the last decades, 30% of RCC patients are diagnosed with metastatic or advanced carcinoma at first visit. It was reported that the 5-year survival for patients is below 10% once malignancy metastasis. RCC patients are high resistance to conventional chemotherapy and radiotherapy. Therefore the surgery is considered as the most effective method for the treatment of localized primary RCC. But there are still high percentages of RCC patients develop metastases after nephrectomy. Thus, the illustration of risk factors involved in chemoresistance of RCC cells will be great helpful for clinical treatment and drug development of RCC.

Chemokines have been revealed to regulate the tumorigenesis and development of RCC. Nowadays, more and more researches suggested that interleukin-6 (IL-6), one of the most commonly researched cytokines in cancer, regulates the metastasis and prognosis of RCC. Cellular studies indicated that primarily cultured RCC cells can secrete increased levels of IL-6. The high levels of IL-6 mRNA and protein were also observed in fresh RCC tissues. Functional studies indicated that IL-6 can increase the invasion and migration of RCC cells and trigger the epithelial-mesenchymal transition (EMT). Clinical data also revealed that serum IL-6 was undetectable in heath people while presented in metastatic RCC patients. Higher serum levels of IL-6 were correlated with worse prognosis outcomes of RCC. All these data suggested the promotion effects of IL-6 on the progression of RCC. As a T-cell-derived cytokine, mechanism researched suggested that IL-6 can trigger the invasion, proliferation and angiogenesis of cancer cells via activation of STAT3 and induction of expression of intercellular adhesion molecule-1 (ICAM-1). The activation of ERK1/2 and Akt also mediated the biologic effects of IL-6 in cancer cells. However, the roles and related mechanisms of chemokines including IL-6 on the development of RCC remain to be further illustrated.

Recently, studies indicated that cytokines particularly interleukins are important for the progression of RCC.

CONTACT Yongjun Wang yongjunwanghb@yeah.net Cardiovascular Department, Hebei Chest Hospital, No. 372 Sheng Li Street, Shijiazhuang, Hebei, 050041, China.
We checked the expression of various interleukins in renal carcinoma cells and compared with human renal proximal tubule epithelial cell line HK-2. The results showed that the expression of IL-6 was significantly upregulated in RCC cells. Further studies indicated that IL-6/STAT3 axis plays an important role in the malignancy and doxorubicin (Dox) sensitivity of RCC cells.

**Materials and methods**

**Cell line and cell culture**

The human RCC cell lines ACHN, A498, Caki-1, and Caki-2, and human renal proximal tubule epithelial cell line HK-2 were purchased from American Type Culture Collection (Manassas, VA) and cultured with DMEM or RPMI 1640 medium with the supplement of 10% fetal bovine serum (FBS, Gibco, NY) and 1% penicillin-streptomycin. Cells were checked for the free of mycoplasma and bacterial contaminants. RCC cells were detached using 0.25% trypsin/0.01% EDTA.

**Real time PCR**

The total RNA was extracted by use of TRIzol reagent (Life Technologies Corporation, Grand Island, NY). Then 1000 ng total RNA was converted to cDNA by using cDNA synthesis kit (Amersham; GE Healthcare, Chalfont, UK) according to the manufacturer’s instructions. Then the mRNA levels of targeted genes were measured by use of a TaqMan Universal PCR Master Mix kit (Applied Biosystems; Thermo Fisher Scientific, Inc.) and analyzed with a LightCycler 480 (Roche Diagnostics, Indianapolis, IN, USA). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control. The data were determined using the $2^{-\Delta\Delta Cq}$ method. The sequences of the various primers were: IL-2, forward, AGA ACT CAA ACC TCT GGA GGA AG, reverse, GCT GTC TCA TCA GCA TAT TCA CAC; IL-4, forward, CAT CGG CT TTT GAA CGA GGT CA, reverse, CTT ATC GAT GAA TCA GGC ATC G; IL-6, forward, ATG GAT GCT ACC AAA CTG GAT, reverse, TGA AGG ACT CTC TG TCT GG; IL-6, forward, ATG GAT GCT ACC AAA CTG GAT, reverse, TGA AGG ACT CTC TG TCT GG; IL-10, forward, CAC CTC AAG AAC ATC CAG AGC T, reverse, CAA GCA GAA CTG AAC TAC CT CG; IL-10, forward, GGT TGC CAA GCC TTA TCG GA, reverse, ACC TGC TCC ACT GCC TTG CT.

**ELISA**

IL-6 in supernatant was analyzed using an ELISA kit (eBioscience, San Diego, CA, USA) according to the manufacturer’s recommendations.

**Western blot analysis**

After treatment, cells were washed and extracted in the lysis buffer. After denatured by boiling in Laemmli buffer, equal amounts of protein (20 μg) were loaded and separated on in 0.1% sodium dodecyl sulfate (SDS), 4–12% gradient acrylamide gels and transferred to nitrocellulose membranes. Then the membranes were blocked with 5% non-fat milk at room temperature for 2 h and immunoblotted with indicated primary antibodies overnight at 4°C. Horseradish peroxidase—conjugated secondary antibodies reacted with the membrane at room temperature for 1 h. Immobilized antibodies were then detected by ECL chemiluminescent reagent (Pierce) with visualized with the ImageQuant LAS 4000 system (GE Healthcare Life Sciences, Uppsala, Sweden).

**Cell transfection**

For transfection, cells were seeded with 70% confluence and transfected with plasmid by use of Lipofectamine 2000 (Invitrogen) or siRNAs by use of Lipofetamine RNAiMAX (Invitrogen) according to the manufacturer’s instructions. The efficiency of knockdown was validated by real time PCR.

**Cell proliferation assay**

The effects of IL-6 on the proliferation of RCC cells were examined by use of WST-8 (Roche Biochemicals, Mannheim, Germany) according to the manufacturer’s instructions. Briefly, cells were cultured in 96 well plates and treated as indicated conditions. Then 10 μl of CCK8 was added into each well at the harvest time and incubated for another 2 hours. The relative cell proliferation was determined by measuring the absorbance of the converted dye at 490 nm. Three independent experiments were performed and untreated cells were used as blank controls.

**Cell migration and invasion**

The effects of IL-6 on the migration and invasion of RCC cells were tested by use of Transwell chambers (8-μm polycarbonate membrane, Costar, Corning Inc.) coated with or without Matrigel (BD Biosciences, Billerica) according to previous studies. Briefly, the top chamber of transwell was loaded with 0.2 ml of 4 × 10^5 cells/ml in serum-free media, and the bottom chamber was loaded with 0.6 ml of medium containing 0.1% FBS. After treated with indicated times, the penetrated cell will be fixed with 75% ethanol, stained with 1% crystal violet solution (Fisher Scientific, PA, USA), and cells were
counted under microscope. Three independent experiments were performed.

**Statistical analysis**

Data were presented as the mean ± SD of 3 independent experiments. The data were analyzed by use of SPSS statistical software version 18.0 (SPSS Inc., Chicago, IL) with *P* < 0.05 was considered statistically significant. Comparisons between 2 groups were done by the unpaired Student’s t-test.

**Results**

**The expression of IL-6 is significantly increased in RCC cells**

Studies indicated that IL-2, IL-4, IL-6, IL-8, and IL-10 were involved in the progression of RCC. We then measured their expression in the human RCC cell lines ACHN, A498, Caki-1, and Caki-2, and human renal proximal tubule epithelial cell line HK-2 cells by qRT-PCRs. The results showed that compared the HK-2 cells, IL-6 was significantly increased in RCC cells, particularly in ACHN and A498 cells (Figure 1A). No similar result was observed for IL-2, IL-4, IL-8, or IL-10. The up regulation of IL-6 in RCC cells was further confirmed by the results of EILISA (Figure 1B) and Western blot analysis (Figure 1C). Collectively, our data suggested that the expression of IL-6 was significantly upregulated in RCC cells.

**Targeted inhibition of IL-6 suppresses the proliferation, migration and invasion of RCC cells**

We then investigated the effect of IL-6 on the proliferation, migration and invasion of RCC cells by use of siRNAs. Our data showed that si-IL-6 can effectively knock down the expression of IL-6 (Figure 2A) and significantly decrease the in vitro proliferation of both ACHN (Figure 2B) and A498 (Figure 2C) cells. In addition, si-IL-6 also can inhibit the migration (Figure 2D) and invasion (Figure 2E) of ACHN and A498 cells. Take together, our data revealed that targeted inhibition of IL-6 by its
specific siRNA can suppress the proliferation, migration and invasion of RCC cells.

IL-6 is involved Dox sensitivity of RCC cells

Chemotherapy has been considered as the major approach for RCC treatment, we therefore investigated the effects of IL-6 on the Dox sensitivity of RCC cells. Our results showed that in IL-6 knocked down ACHN cells, the IC50 value of Dox (121 nM) was significant (p<0.05) lower than that in si-NC transfectd ACHN cells (350 nM) (Figure 3A). Similar data were also observed in A498 cells (Figure 3B). In addition, the recombination IL-6 can attenuate the inhibition effects of Dox on the proliferation of both ACHN (Figure 3C) and A498 (Figure 3D) cells. These data suggested that the increased expression of IL-6 is involved in Dox sensitivity of RCC cells.

STAT3 is involved in IL-6 regulated Dox sensitivity of RCC cells

It was indicated that STAT3 signals mediated the biologic effects of IL-6 in various cancer cells.18 We then tested the role of STAT3 in IL-6 regulated Dox sensitivity of RCC cells. Firstly, we found that si-IL-6 can decrease the phosphorylation of STAT-3 in both ACHN and A498 cells (Figure 4A). We then overexpressed the STAT-3 in ACHN and A498 cells (Figure 4B). Over expression of STAT3 can significantly trigger the proliferation of ACHN and A498 cells (Figure 4C). In addition, overexpression of SATA3 also reversed si-IL-6 induced increase of Dox sensitivity of ACHN and A498 cells (Figure 4D). Take together, our data revealed that STAT3 is involved in IL-6 regulated Dox sensitivity of RCC cells.

STAT3 is also involved in IL-6 regulated migration and invasion of RCC cells

Considering that IL-6 can also regulate the migration and invasion of RCC cells, we further evaluate whether STAT3 is also involved in this progression. Our results revealed that overexpression of STAT3 can trigger the migration of both ACHN and A498 cells (Figure 5A). Similarly, overexpression of STAT3 also increased the invasion of both ACHN and A498 cells (Figure 5B). In addition, overexpression of STAT3 also attenuated the inhibition effects of si-IL-6 on the migration (Figure 5C) and invasion (Figure 5D) of ACHN cells. Similar data also observed in A498 cells (data not shown). Collectively, it suggested that STAT3 is also
involved in IL-6 regulated migration and invasion of RCC cells.

**Activation of ERK1/2 participates IL-6 regulated Dox sensitivity of RCC cells**

Recent studies indicated that the activation of ERK1/2 and Akt also mediated the biologic effects of IL-6 in cancer cells. Our results showed that recombination IL-6 can trigger the phosphorylation of both ERK1/2 and Akt in ACHN cells (Figure 6A). However, only ERK1/2 inhibitor PD98059, while not PI3K/Akt inhibitor LY294002, can attenuate IL-6 induced proliferation of ACHN cells (Figure 6B). In addition, ERK1/2 inhibitor PD98059 can synergistically increased the Dox sensitivity of si-IL-6 transfected ACHN cells (Figure 6C), while PI3K/Akt inhibitor LY294002 had no similar effects. These results suggested that activation of ERK1/2 participates IL-6 regulated Dox sensitivity of RCC cells.

**Discussion**

IL-6, which is initially identified as a critical regulator of the immune response, has been suggested to promote the progression of various cancers. Our present study revealed that the expression of IL-6 was significantly increased in RCC cells compared with the human renal proximal tubule epithelial cell line HK-2. Targeted inhibition of IL-6 can suppress the proliferation, migration and invasion of ACHN and A498 cells. In addition, silencing of IL-6 increased the Dox sensitivity of RCC cells, while recombination IL-6 can attenuate the toxicity of Dox. STAT3 mediated IL-6 regulated Dox sensitivity, migration and invasion of RCC cells. Furthermore,
ERK1/2 is also involved in IL-6 regulated Dox sensitivity of ACHN cells. Our data suggested that IL-6/STAT3 signals acts as important role in the progression and Dox sensitivity of RCC.

Our data revealed that the high expression of IL-6 can trigger the proliferation, migration, invasion and Dox resistantance of RCC cells. IL-6 has been supposed to be one of the most critical cytokines of growth of various cancers such as breast cancer and cholangiocarcinoma. Our results revealed that IL-6, while not IL-2, 4, 8 or 10, was significantly increased in RCC cells, which is consistent with previous study that IL-6 functions as an in vitro autocrine growth factor in RCC. IL-6 has been supposed to be one of the most critical cytokines of growth of various cancers such as breast cancer and cholangiocarcinoma. Our results revealed that IL-6, while not IL-2, 4, 8 or 10, was significantly increased in RCC cells, which is consistent with previous study that IL-6 functions as an in vitro autocrine growth factor in RCC. IL-6 can trigger the proliferation of renal cancer ACHN and 769P cells, which is consistent with our present study that knocked down of IL-6 can suppress the proliferation of ACHN and A498 cells. IL-6 can trigger the migration and invasion of breast cancer cells via induction of epithelial–mesenchymal transition (EMT) of breast cancer cells. The autocrine production of IL-6 can also lead to multidrug resistance in cancer cells. Over expression of IL-6 can increase the paclitaxel resistance of human osteosarcoma cells and lapatinib resistance in breast cancer cells. Collectively, our present study, together with published literatures, revealed that increased levels of IL-6 can trigger the malignancy and Dox resistance of RCC.

STAT3 is the major downstream signal mediator of IL-6 signals. Numerous studies indicated that IL-6/STAT3 signals promote the growth and development of cancers. Our present study revealed that overexpression of STAT3 can trigger the proliferation, migration and invasion, and reversed si-IL-6 induced increase of Dox sensitivity of RCC cells. This is consistent with

Figure 4. STAT3 is involved in IL-6 regulated Dox sensitivity of RCC cells. (A) ACHN or A498 cells were transfected with si-NC or si-IL-6 for 24 h; (B) ACHN or A498 cells were transfected with pcDNA 3.1 (vector) or pcDNA/STAT3 (STAT3) for 24 h; (C) ACHN or A498 cells were transfected with pcDNA 3.1 (vector) or pcDNA/STAT3 (STAT3) for the indicated times, the proliferation of RCC cells were measured by CCK-8 kit; (D) Cells transfected with indicated conditions were further treated with vehicle or 100 nM Dox for another 24 h, the proliferation of RCC cells were measured by CCK-8 kit. *p < 0.05 compared with the si-NC si-IL-6 + vector + Dox.
Figure 5. STAT3 is also involved in IL-6 regulated migration and invasion of RCC cells. ACHN or A498 cells were transfected with pcDNA 3.1 (vector) or pcDNA/STAT3 (STAT3) for 48 h, then the migration (A) or invasion (B) effects were measured by use of transwell assays; ACHN cells were transfected with in the indicated treatments of 48 h, the migration (A) or invasion (B) effects were measured by use of transwell assays. *p < 0.05 compared with the control, # p < 0.05 compared with the si-IL-6 vector group.

Figure 6. Activation of ERK1/2 participates IL-6 regulated Dox sensitivity of RCC cells. (A) ACHN cells were treated with recombination IL-6 (40 ng/ml) for the indicated times, the total and phosphorylation of ERK1/2 and Akt were measured by western blot analysis; (B) ACHN cells were treated with recombination IL-6 (40 ng/ml) in the present or absence of ERK inhibitor PD985059 (PD, 10 μM) or Akt inhibitor LY294002 (LY, 10 μM) for 48 h, the cell proliferation was measured by CCK-8 kit; (C) ACHN cells transfected with si-IL-6 were treated with increasing concentrations of Dox with ERK inhibitor PD985059 (PD, 10 μM) or Akt inhibitor LY294002 (LY, 10 μM) for 48 h, the cell proliferation was measured by CCK-8 kit. *p < 0.05 compared with the IL-6 group.
previous studies that inhibition of STAT3 can increase the Dox sensitivity of metastatic breast cancer cells.29 In addition, STAT3 also mediated the IL-6 induced proliferation of RCC ACHN and 769P cells.22 The activation of STAT3 has been reported to protect tumor cells from apoptosis and promote the metastasis and angiogenesis of tumors.18 While inhibition of STAT3 signals can induce apoptosis and suppress metastasis of RCC cells.30 Our study also revealed that activation of ERK1/2 also participated IL-6 regulated Dox sensitivity of RCC cells, which was evidenced by the results that IL-6 increased the phosphorylation of ERK1/2 and ERK1/2 inhibitor can reverse IL-6 induced proliferation of RCC cells. This is consistent with previous studies that IL-6 can increase the phosphorylation of ERK1/2 in various cells.31

Take together, our data showed that IL-6 is over-expressed in RCC cells and trigger the proliferation, migration, invasion and Dox resistance of RCC cells. The activation of STAT3 and ERK1/2 mediated the promotion effects of IL-6 on RCC progression. Therefore targeted inhibition of IL-6 signals might be a helpful approach for RCC treatment.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

References

[1] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA Cancer J Clin 2015; 65:5-29; PMID:25559415; https://doi.org/10.3322/caac.21254

[2] Haake SM, Rathmell WK. Renal cancer subtypes: Should we be lumping or splitting for therapeutic decision making? Cancer 2017; 123(2):200-9; PMID:27861752.

[3] Courthod G, Tucci M, Di Maio M, Scaglotti GV. Papillary renal cell carcinoma: A review of the current therapeutic landscape. Crit Rev Oncol Hematol 2015; 96:100-12; PMID:26052049; https://doi.org/10.1016/j.critrevonc.2015.05.008

[4] Ciccarese C, Massari M, Heng DYC, Sotte V, Brunelli M, Conti A, Cheng L, Lopez-Beltran A, Scarpelli M, et al. New molecular targets in non clear renal cell carcinoma: An overview of ongoing clinical trials. Cancer Treat Rev 2015; 41:614-22; PMID:26036356; https://doi.org/10.1016/j.ctrv.2015.05.006

[5] van Sproonsen DJ, de Weijer KJM, Mulders PFA, De Mulder PHM. Novel treatment strategies in clear-cell metastatic renal cell carcinoma. Anticancer Drugs 2005; 16:709-17; PMID:16027518; https://doi.org/10.1097/01.cad.0000167901.58877.a3

[6] Parlibar JS, Tunuguntla HSGR. Role of chemokines in renal cell carcinoma. Rev Urol 2014; 16:118-21; PMID:25337041.

[7] Hrab M, Olek-Hrab K, Antczak A, Kwisz Z, Milecki T. Interleukin-6 (IL-6) and C-reactive protein (CRP) concentration prior to total nephrectomy are prognostic factors in localized renal cell carcinoma (RCC). Rep Pract Oncol Radiother 2013; 18:304-9; PMID:24416568; https://doi.org/10.1016/j.rpor.2013.06.002

[8] Takenawa J, Kaneko Y, Fukumoto M, Fukatsu A, Hirano T, Fukuyama H, Nakayama H, Fujita J, Yoshida O. Enhanced expression of Interleukin-6 in primary human renal cell Carcinomas. J Natl Cancer Inst 1991; 83:1668-72; PMID:1749019; https://doi.org/10.1093/jnci/83.22.1668

[9] Chuang M-J, Sun K-H, Tang S-J, Deng M-W, Wu Y-H, Sung J-S, Cha TL, Sun GH. Tumor-derived tumor necrosis factor-alpha promotes progression and epithelial-mesenchymal transition in renal cell carcinoma cells. Cancer Sci 2008; 99:905-13; PMID:18294286; https://doi.org/10.1111/j.1349-7006.2008.00756.x

[10] Favaro D, Santarosa M, Quaia M, Galligioni E. Interleukin-6 and soluble intercellular adhesion molecule-1 in renal cancer patients and cultured renal cancer cells. Urol Oncol Semin Orig Invest 1997; 3:51-8; https://doi.org/10.1016/S1078-1439(97)00036-7

[11] Dolcet Q, Schaez A, Faucher C, Lepage E, Wautier J-L, Richard F, Cabane J. Tumor necrosis factor-α, interleukin-1β and interleukin-6 in patients with renal cell carcinoma. Eur J Cancer 1994; 30:162-7; https://doi.org/10.1016/0959-8049(94)90079-5

[12] Tu B, Du L, Fan Q-M, Tang Z, Tang T-T. STAT3 activation by IL-6 from mesenchymal stem cells promotes the proliferation and metastasis of osteosarcoma. Cancer Lett 2012; 325:80-8; PMID:22743617; https://doi.org/10.1016/j.canlet.2012.06.006

[13] Lin Y-M, Chang Z-L, Liao Y-Y, Chou M-C, Tang C-H. IL-6 promotes ICAM-1 expression and cell motility in human osteosarcoma. Cancer Lett 2013; 328:135-43; PMID:22939995; https://doi.org/10.1016/j.canlet.2012.08.029

[14] Frampton G, Invernizzi P, Bernuzzi F, Pae HY, Quinn M, Horvat D, Galindo C, Huang L, McMillin M, Cooper B, et al. Interleukin-6-driven progranulin expression increases cholangiocarcinoma growth by an Akt-dependent mechanism. Gut 2012; 61:268-77; PMID:22068162; https://doi.org/10.1136/gutjnl-2011-300643

[15] Zhang B, Fenton RG. Proliferation of IL-6-independent multiple myeloma does not require the activity of extracellular signal-regulated kinases (ERK1/2). J Cell Physiol 2002; 193:42-54; PMID:1209879; https://doi.org/10.1002/jcp.10148

[16] Kamińska K, Czarnecka AM, Escudier B, Lian F, Szczyluk C. Interleukin-6 as an emerging regulator of renal cell cancer. Urol Oncol 2015; 33:476-85; PMID:2629624; https://doi.org/10.1016/j.urolonc.2015.07.010

[17] Gao Y, Ma X, Yao Y, Li H, Fan Y, Zhang Y, Zhao C, Wang L, Ma M, Lei Z, et al. miR-155 regulates the proliferation and invasion of clear cell renal cell carcinoma cells by targeting E2F2. Oncotarget 2016; 7:20324-37; PMID:26967247.

[18] Yu H, Pardoll D, Jove R. STATs in cancer inflammation and immunity: A leading role for STAT3. Nat Rev Cancer 2009; 9:798-809; PMID:19851315; https://doi.org/10.1038/nrc2734

[19] Heinrich PC, Behrmann I, Haan S, Hermanns HM, Müller-Newen G, Schaper F. Principles of interleukin (IL)-6-type cytokine signalling and its regulation.
Biochem J 2003; 374:1-20; PMID:12773095; https://doi.org/10.1042/bj20030407

[20] Knüpf H, Preiß R. Significance of interleukin-6 (IL-6) in breast cancer (review). Breast Cancer Res Treat 2007; 102:129-35; PMID:16927176; https://doi.org/10.1007/s10549-006-9328-3

[21] Miki S, Iwano M, Miki Y, Yamamoto M, Tang B, Yokokawa K, Sonoda T, Hirano T, Kishimoto T. Interleukin–6 (IL–6) functions as an in vitro autocrine growth factor in renal cell carcinomas. FEBS Lett 1989; 250:607-10; PMID:2787758; https://doi.org/10.1016/0014-5793(89)80805-1

[22] Horiguchi A, Oya M, Marumo K, Murai M. STAT3, but not ERKs, mediates the IL-6-induced proliferation of renal cancer cells, ACHN and 769P. Kidney Int 2002; 61:926-38; PMID:11849447; https://doi.org/10.1046/j.1523-1755.2002.00206.x

[23] Sullivan NJ, Sasser AK, Axel AE, Vesuna F, Raman V, Ramírez N, Oberegyn TM, Hall BM. Interleukin-6 induces an epithelial–mesenchymal transition phenotype in human breast cancer cells. Oncogene 2009; 28:2940-7; PMID:19581928; https://doi.org/10.1038/onc.2009.180

[24] Walter M, Liang S, Ghosh S, Hornsby PJ, Li R. Interleukin 6 secreted from adipose stromal cells promotes migration and invasion of breast cancer cells. Oncogene 2009; 28:2745-55; PMID:19483720; https://doi.org/10.1038/onc.2009.130

[25] Conze D, Weiss L, Regen PS, Bhushan A, Weaver D, Johnson P, Rincón M. Autocrine production of Interleukin 6 causes multidrug resistance in breast cancer cells. Cancer Res 2001; 61:8851-8. PMID:11751408.

[26] Duan Z, Lamendola DE, Penson RT, Kronish KM, Seiden MV. Overexpression of IL-6 but not IL-8 increases paclitaxel resistance of U-2OS human Osteosarcoma cells. Cytokine 2002;17:234-42; PMID:12027404; https://doi.org/10.1002/cyt.2001.1008

[27] Huang W-C, Hung C-M, Wei C-T, Chen T-M, Chien P-H, Pan H-L, Lin YM, Chen YJ. Interleukin-6 expression contributes to lapatinib resistance through maintenance of stemness property in HER2-positive breast cancer cells. Oncotarget 2016;7:62352-63. PMID:27694691.

[28] Lee H, Herrmann A, Deng J-H, Kujawski M, Niu G, Li Z, Forman S, Jove R, Pardoll DM, Yu H. Persistently activated Stat3 maintains constitutive NF-kappaB activity in tumors. Cancer Cell 2009; 15:283-93; PMID:19345327; https://doi.org/10.1016/j.ccr.2009.02.015

[29] Gariboldi MB, Ravizza R, Molteni R, Osella D, Gabano E, Monti E. Inhibition of Stat3 increases doxorubicin sensitivity in a human metastatic breast cancer cell line. Cancer Lett 2007; 258:181-8; PMID:17920763; https://doi.org/10.1016/j.canlet.2007.08.019

[30] Fang Z, Tang Y, Fang J, Zhou Z, Xing Z, Guo Z, Guo X, Wang W, Jiao W, Xu Z, et al. Simvastatin inhibits renal cancer cell growth and metastasis via AKT/mTOR, ERK and JAK2/STAT3 pathway. PLoS One 2013; 8(5):e62823; http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3656850/.

[31] Wu T-H, Lin C-H. IL-6 mediated alterations on immobile behavior of rats in the forced swim test via ERK1/2 activation in specific brain regions. Behav Brain Res 2008; 193:83-91; PMID:18573547; https://doi.org/10.1016/j.bbr.2008.05.009