



## Interleukin-6 induces drug resistance in renal cell carcinoma

メタデータ	言語: English 出版者: The Fukushima Society of Medical Science 公開日: 2018-12-18 キーワード (Ja): キーワード (En): renal cell carcinoma, IFN, IL-6, SOCS 3, TKI 作成者: Ishibashi, Kei, Koguchi, Tomoyuki, Matsuoka, Kanako, Onagi, Akifumi, Tanji, Ryo, Takinami-Honda, Ruriko, Hoshi, Seiji, Onoda, Mitsutaka, Kurimura, Yoshimasa, Hata, Junya, Sato, Yuichi, Kataoka, Masao, Ogawa, Soichiro, Haga, Nobuhiro, Kojima, Yoshiyuki メールアドレス: 所属:
URL	<a href="https://fmu.repo.nii.ac.jp/records/2001962">https://fmu.repo.nii.ac.jp/records/2001962</a>

[Review]

## Interleukin-6 induces drug resistance in renal cell carcinoma

Kei Ishibashi, Tomoyuki Koguchi, Kanako Matsuoka, Akifumi Onagi,  
Ryo Tanji, Ruriko Takinami-Honda, Seiji Hoshi, Mitsutaka Onoda,  
Yoshimasa Kurimura, Junya Hata, Yuichi Sato, Masao Kataoka,  
Soichiro Ogawsa, Nobuhiro Haga and Yoshiyuki Kojima

*Department of Urology, Fukushima Medical University School of Medicine, Fukushima, Japan*

(Received August 7, 2018, accepted September 6, 2018)

### Abstract

Metastatic renal cell carcinoma (mRCC) is a tumor entity with poor prognosis due to limited therapy options. Tyrosine kinase inhibitors (TKIs), the novel targeted agents have been used for the treatment of mRCC and have shown efficacy. Interferon (IFN)- $\alpha$  is also one of the most frequently used agents in immunotherapy. However, drug resistance needs to be overcome to achieve a sufficiently positive effect. Interleukin-6 (IL-6), which induce suppressor of cytokine signaling-3 (SOCS3) expression, is one of the factors associated with poor prognosis of patients with renal cell carcinoma (RCC). To analyze the influence of IL-6 in drug resistance of RCC, anti-IL-6 receptor antibody was used in combination with IFN or TKIs. The SOCS3 mRNA expression level was significantly increased by IFN- $\alpha$  stimulation in 786-O RCC cells which were resistant to IFN, but not in ACHN cells that were sensitive to IFN. The overexpression of SOCS3 by gene transfection in ACHN significantly inhibited the growth-inhibitory effect of IFN- $\alpha$ . An *in vivo* study demonstrated that co-administration of SOCS3-targeted siRNA promoted IFN- $\alpha$ -induced cell death and growth suppression in 786-O cell xenograft. SOCS3 could be a key component in the resistance to interferon treatment of renal cell carcinoma. Because SOCS3 is rapidly up-regulated by IL-6 and a negative regulator of cytokine signaling, IL-6 expression on RCC cells was also analyzed and the 786-O cells showed the high level of IL-6 mRNA expression under the condition of interferon stimulation. IL-6R antibody, tocilizumab, significantly suppressed cell proliferation in 786-O cells by interferon stimulation accompanied with phosphorylation of STAT1 and inhibited SOCS3 expression. The *in vivo* effects of combination therapy with tocilizumab and interferon showed significant suppression of 786-O tumor growth in a xenograft model. We also hypothesized that TKI resistance and IL-6 secretion are causally connected. And we found that 786-O RCC cells secrete high IL-6 levels after low dose stimulation with the TKIs sorafenib, sunitinib and pazopanib, inducing activation of AKT-mTOR pathway, NF $\kappa$ B, HIF-2 $\alpha$  and VEGF expression. Tocilizumab neutralizes the AKT-mTOR pathway activation and results in reduced proliferation. A combination therapy with tocilizumab and TKI suppresses 786-O tumor growth and inhibits angiogenesis *in vivo* more efficient than TKI alone. Our findings suggest that IL-6 could induce drug resistance on RCC, and combination therapy of IL-6R inhibitors and IFN/TKIs may represent a novel therapeutic approach for RCC treatment.

**Key words :** renal cell carcinoma, IFN, IL-6, SOCS 3, TKI

## Introduction

Renal cell carcinomas (RCC) account for about 85 percent of renal cancers and a quarter of the patients present with advanced disease, including locally invasive or metastatic renal cell carcinoma<sup>1</sup>. As a therapeutic strategy against RCC, surgery is the standard treatment for localized disease. However, its role in the presence of distant metastases is limited. Molecular targeted agents, vascular endothelial growth factor receptor tyrosine kinase inhibitors (TKIs), is a standard care for advanced RCC. TKIs have been used for the treatment of advanced RCC and have shown efficacy against metastatic RCC<sup>2-4</sup>. Recently, nivolumab, a programmed death 1 (PD-1) checkpoint inhibitor, was approved for previously treated patients with advanced RCC, based on the superior overall survival of nivolumab versus everolimus<sup>5</sup>. In the era of the recent progression for the treatment option, immunotherapy seems to have a minimal role in the management of advanced RCC. However, the value of immune-therapy for RCC is supported by reports of infrequent complete regression of metastatic disease with cytokine therapies and about 14 percent of cases of metastatic clear-cell renal carcinoma respond to interferon (IFN)- $\alpha$  alone. Thus, IFN- $\alpha$  is still one of the most frequently used agents in immunotherapy against metastatic or recurrent RCC, especially for lung metastasis<sup>1</sup>. Many reports have indicated that IFN- $\alpha$  therapy improved the survival of RCC patients and can lead to a complete response<sup>6,7</sup>. Although IFN- $\alpha$  can be used as an alternative in non-responders to targeted therapy, its benefits are limited due to drug resistance. Studies of drug resistance on RCC will provide clues for future strategies in treatment of advanced RCC.

### Systemic treatment in advanced renal cell carcinoma

RCC is characterized by its poor response to conventional chemotherapy and radiation therapy because of its unique biology. However, understanding biologic feature of RCC has made it possible to advance in systemic therapy against advanced RCC.

Cytokines, including IFN- $\alpha$  and interleukin 2 (IL-2), has been the standard treatment as non-specific immunotherapy. They were established as the first effective immunotherapy and were used in combinations as well as monotherapy. As for IFN- $\alpha$ , the efficacy and safety were established in

several studies and its response rate varies of 5 to 27%<sup>8,9</sup>. Combination therapy with IFN- $\alpha$  and IL-2 was confirmed to be effective for renal cell carcinoma patients with lung metastasis<sup>10,11</sup>. The median survival time of advanced RCC patients in Japanese population was longer than that of previous studies and cytokine-based therapy might be one of the factors that improve the prognosis of RCC patients<sup>12</sup>.

Therapy targets the vascular endothelial growth factor (VEGF) and the mammalian target of rapamycin (mTOR) pathways represents the standard of care in metastatic RCC. The multi-targeted tyrosine kinase inhibitors (TKI) lead to clearly prolonged overall and progression-free survival<sup>13</sup>. Sorafenib inhibits vascular endothelial growth factor (VEGF) receptors VEGFR-2, VEGFR-3, the platelet-derived growth factor receptor family which play key roles in tumor progression and angiogenesis<sup>14</sup>. Sunitinib is a selective inhibitor of VEGF-R types 1 to 3, PDGFR- $\alpha$ , and PDGFR- $\beta$ <sup>15</sup>. Pazopanib also inhibits all the VEGFR subtypes and the PDGFR subtypes<sup>16</sup>. Axitinib is an oral, potent, and selective inhibitor of VEGF receptors 1, 2, and 3<sup>17</sup> and showed promising effect against advanced RCC<sup>18</sup>. However, despite the development of many types of TKIs, their effects are still limited and have been shown to be not curative<sup>19</sup>. The treatment has been associated with the development of resistance after a median of 6-15 months<sup>20</sup>. Mammalian target of rapamycin (mTOR) regulates cell growth and proliferation. Akt-mTOR pathway is another target for the treatment against advanced RCC. The mTOR inhibitors, everolimus and temsirolimus, interfere directly with it by acting on mTOR, reducing the activity of the effector molecules S6K1 and 4EBP1. As a consequence, they inhibit cell proliferation, growth and survival<sup>21,22</sup>.

Nivolumab, a programmed death 1 (PD-1) checkpoint inhibitor, restores T-cell immune activity. Nivolumab demonstrated promising antitumor activity with a manageable safety profile<sup>5</sup>. Patients with 1 or 2 prior anti-angiogenic therapies, which is defined as prior VEGFR-targeted therapy, were randomized to receive either nivolumab or mTOR inhibitor everolimus. As consequent, overall survival was longer with nivolumab than with everolimus (25.0 vs. 19.6 months, HR 0.73 ;  $p = 0.002$ )<sup>23</sup>. These results implies the importance of anti-tumor immunity in RCC treatment.

### Interleukin-6 (IL-6) signaling and cancer

IL-6-STAT3 pathway has a key role in the

growth and development of many human cancers. The IL-6-STAT3 axis activates target genes involved in cell survival, and proliferation. IL-6 is produced by many types of cells which located within the tumour microenvironment, including the tumour cells themselves<sup>24</sup>. IL-6 binds to the membrane-bound IL-6 receptor (IL-6R) on tumour cells to induce the expression of STAT3 target genes. The STAT3 promote angiogenesis and tumor invasion through VEGF and matrix metalloproteinases expression<sup>25-27</sup>. The IL-6 can also activate the PI3K/AKT/mTOR and RAS/RAF/MEK/ERK pathways<sup>28,29</sup>. IL-6-induced activation of Akt/mTOR consequently activates its downstream targets p70S6 kinase (p70S6K), 40S ribosomal protein S6 (S6RP) and the eukaryotic initiation factor 4E binding protein-1 (4EBP1), that control mRNA translation and protein synthesis<sup>30</sup>. IL-6-induced activation of AKT/mTOR is involved in protection against apoptosis and in enhanced proliferation in cancer cells<sup>31,32</sup>. Furthermore, STAT3 promote IL-6 expression. Thus, the IL-6 gene expression then results in a feedforward autocrine feedback loop<sup>33,34</sup>. Hyperactivation of STAT3 signalling occurs in many type of human cancers and correlates with a poor patient outcomes<sup>35-38</sup>. Multiple studies have documented high levels of IL-6 in the serum of patients with renal cell carcinomas and that it is associated with a poor prognosis<sup>39,40</sup>. Moreover, there is a study which reported a trend for a higher IL-6 level to be associated with the failure to complete immunotherapy and with poorer cancer-specific survival in the patients with pretreatment systemic inflammatory response<sup>41</sup>. These results of the studies imply that treatments that target the IL-6/STAT3 pathway in patients with cancer are poised to provide therapeutic benefit.

### SOCS3 affects susceptibility of renal cell carcinoma to IFN- $\alpha$

IFN- $\alpha$  is historically one of the most frequently used agents in immunotherapy against metastatic or recurrent RCC. Although this agent can lead to a complete response<sup>6</sup> and can be used as an alternative in non-responders to targeted therapy, its benefits are limited due to drug resistance. IFN- $\alpha$  can exert a direct antiproliferative response by modulating the expression of proteins that control cell cycle. JAK/STAT pathway is one of the most important signal transduction cascades in IFN signaling. On the other hand, suppressor of cytokine signaling (SOCS) protein family is known to act as negative

regulators of IFN- $\alpha$  signaling by inhibiting the JAK/STAT pathway<sup>42</sup>. Some RNA virus escapes the innate antiviral response, generally type I IFN response, by inducing SOCS expression in epithelial cells<sup>43</sup>. IFN-gamma-induced high SOCS gene expression in murine colon carcinoma cells significantly interferes with the antiproliferative effect of IFN-gamma<sup>44</sup>. Eight SOCS family proteins have been described, CIS (cytokine-inducible SH2 domain-containing protein) and SOCS1 to SOCS7<sup>42</sup>. Among the SOCS family, SOCS1 and SOCS3 have been identified as inhibitors of the IFN-mediated JAK-STAT signaling pathways<sup>45</sup>. Thus, we quantified the mRNA expression levels of the SOCS family including CIS, SOCS1 and SOCS3, in the RCC cell lines after IFN treatment. After stimulation with IFN- $\alpha$ , CIS, SOCS1 and SOCS3 mRNA expression increased in RCC cells, ACHN and 786-O, compared with that in pre-treated cells. Among the SOCS-mRNA, SOCS3-mRNA was significantly higher in 786-O than in ACHN cells ( $p < 0.01$ ), although no significant differences in CIS and SOCS1 mRNA expression level were observed. To determine the role of SOCS3 in IFN- $\alpha$  susceptibility, siRNA targeting SOCS3 was used for knock-down of the SOCS3 expression. As a result, the knock down of SOCS3 in 786-O cells, which did not show susceptibility to IFN, induced a growth inhibitory effect of IFN- $\alpha$ , whereas no effect was observed on transfection with the negative control siRNA ( $p < 0.01$ ). On the contrary, transfection of SOCS3 using pCI-neo expression vector to ACHN cells, which showed susceptibility to IFN, induced decreased growth inhibitory effect of INF compared with mock transfectant and non-treated ACHN cells ( $p < 0.01$ ). Western blot analysis revealed that phosphorylation level of STAT-1 was higher in 786-O cells treated with SOCS3 siRNA than in the control siRNA-treated cells. The effect of SOCS3 knockdown on IFN- $\alpha$  sensitivity was also observed in *in vivo* xenograft athymic mouse model. The growth of the SOCS3 siRNA-treated tumors was retarded in comparison with those in the negative control-siRNA group and non-treated mice. Immunohistochemical examination showed an increased number of apoptotic cells, lymphocyte infiltration and focal fibrosis in the tumor treated with SOCS3 siRNA and IFN. Taken together, the inhibition of SOCS3 expression enhanced STAT1 activation and anti-tumor activity of IFN-alpha, both *in vitro* and *in vivo*, in a human IFN- $\alpha$ -resistant RCC cell line in which SOCS3 mRNA is over-expressed<sup>46</sup>.

### Impact of IL-6 on susceptibility of renal cell carcinoma to IFN- $\alpha$

IL-6 is one of the factors associated with poor prognosis of patients with RCC. IL-6 has widespread effects on hematopoietic lineages and is considered to be a key mediator of anemia of inflammation<sup>47)</sup>. Low serum hemoglobin is a known risk factor for short survival in RCC patients<sup>48)</sup>. IL-6 is produced by multiple cell types including tumor-infiltrating immune cells, stromal cells, and the tumor cells themselves<sup>29,33)</sup>. In response to IL-6 stimulation, phosphorylated STAT3 molecules dimerize and enter the nucleus. SOCS3 is also rapidly up-regulated by IL-6 and acts as a classical feedback inhibitor of cytokine signaling<sup>28,49)</sup>. The silencing of SOCS3 expression is one possible strategy to restore sensitivity to IFN- $\alpha$ -resistant cells. We therefore investigated the role of IL-6 on the IFN resistance in RCC cells. First, we analyzed whether IFN- $\alpha$  altered IL-6 and SOCS3 expression in RCC cells by IFN stimulation. Five human RCC cell lines including ACHN and 786-O were used for the experiment. Consequently, we found that all RCC cell lines induce both IL-6 and SOCS3 mRNA expression by IFN treatment. Among them, the increases in SOCS3 and IL-6 expression were highest in 786-O cells which is IFN resistance cells and lowest in IFN sensitive ACHN cells. The expression levels of IL-6 and SOCS3 were significantly higher in 786-O than in ACHN cells. Likewise, IFN- $\alpha$ -induced IL-6 secretion into the culture medium was highest in 786-O cells and lowest in ACHN cells, as observed for mRNA expression levels. To confirm that the secretion of IL-6 and SOCS3 up-regulation were due to IFN- $\alpha$  stimulation, we used siRNAs targeting IFN alpha receptor (IFNAR), IL-6 and IL-6 receptor (IL-6R). The mRNA expression of IL-6 and SOCS3 after IFN- $\alpha$  treatment was significantly decreased when the IFNAR was knocked down by siRNA. SOCS3 mRNA expression was decreased when IL-6 signaling was knocked down by IL-6-siRNA or IL-6R-siRNA. RCC cells, especially IFN resistance 786-O cells, secreted IL-6 by IFN stimulation which lead SOCS3 expression. Accordingly, we determined the growth inhibitory effect of IFN- $\alpha$  under the blockade of IL-6 signaling by the antihuman IL-6R antibody, tocilizumab, in 786-O cells. Tocilizumab is a humanized antihuman IL-6R antibody that binds to the IL-6-binding site of human IL-6R and inhibits IL-6 signaling. Tocilizumab is therapeutically effective against chronic inflammatory diseases like rheumatoid ar-

thritis and Crohn's disease<sup>50)</sup>. Consequently, we found that antihuman IL-6R antibody inhibits activation of STAT3 in 786-O renal cell carcinoma cells. Phosphorylation level of STAT1 was increased with the simultaneous use of tocilizumab in 786-O cells. A significant IFN- $\alpha$ -induced growth inhibitory effect was observed when the tocilizumab was added to 786-O cells. Contrary, in ACHN cells, the growth inhibitory effect of IFN- $\alpha$  was significantly decreased with reduced phosphorylation of STAT1 when IL-6 and IFN- $\alpha$  were given simultaneously. *In vivo* effect of combination therapy with tocilizumab and IFN- $\alpha$  was confirmed using nude mice xenograft model in 786-O cells which were IFN resistant. Taken together, IL-6 is induced by IFN- $\alpha$  in RCC cells, consequently up-regulating SOCS3 expression which leads to resistance to antiproliferative effect of IFN- $\alpha$  through inhibiting phosphorylation of STAT1. Inhibition of IL-6 signaling by IL-6R antibody tocilizumab enhanced the anti-tumor activity of IFN- $\alpha$  in a human IFN- $\alpha$ -resistant RCC cell line in which IL-6 was induced by IFN- $\alpha$ <sup>51)</sup>.

### Overriding TKI resistance of renal cell carcinoma by combination therapy with tocilizumab

Currently, therapy targets the vascular endothelial growth factor (VEGF) and the mammalian target of rapamycin (mTOR) pathways represents the standard of care in metastatic RCC. Each of these drugs offers significant benefit compared with previous therapeutic standards. Multitargeted tyrosine kinase inhibitors (TKIs) lead to clearly prolonged overall and progression-free survival<sup>13)</sup>. Sorafenib inhibits VEGFR-2, VEGFR-3, the platelet-derived growth factor receptor (PDGFR) family as well as both C-RAF and B-RAF<sup>14)</sup>. Sunitinib which is employed in first-line treatment of advanced RCC<sup>52)</sup> inhibits VEGF-R types 1 to 3, PDGFR- $\alpha$ , and PDGFR- $\beta$ <sup>15)</sup>. Pazopanib is also used in first-line therapy<sup>52)</sup> and inhibits all the VEGFR subtypes and the PDGFR subtypes. However, despite the development of many types of TKIs, unfortunately, their effects are still limited and have been shown to be not curative<sup>19)</sup>. Although the mechanism of TKI resistance is not clear as yet, the finding that IL-6 seems to be involved in the development of resistance<sup>53,54)</sup> suggests that cytokines are important in this process. The IL-6/JAK/STAT3 pathway has a key role in the growth and development of many human cancers, and these molecules are associated with angiogenesis through VEGF ex-

pression<sup>55-57</sup>). We therefore analyzed the impact of IL-6 during TKI treatment of RCC cells. The human RCC cell lines 786-O, A489, Caki1 and Caki2 were used for this study. The RCC cells were treated by three kinds of TKIs, sorafenib, sunitinib and pazopanib. Final concentrations of TKIs were 0.5, 1.0, 5.0, 10.0  $\mu\text{M}$  for sorafenib and sunitinib and 1.0, 5.0, 10.0, 50.0  $\mu\text{M}$  for pazopanib. The supernatant was collected 1, 2, and 24 hours after TKIs stimulation, and the amount of cytokine production (IL-6, VEGF, IL-1ra, IL-17, IL-19, IL-23, IL-18BP, Leptin, HGF, Cript-1, HB-EGF, EGF) was measured by VersaMAP Development System (R&D systems, Minneapolis, MN, USA). Consequently, we found that IL-6 and VEGF secretion by 786-O cells were remarkably enhanced after TKI treatment even at a low concentration. Because all the TKIs induced IL-6 expression in 786-O cells, we studied the impact of TKI stimulation on the associated IL-6 signaling pathway. Western blot analysis revealed that a concentration dependent enhanced phosphorylation of AKT, mTOR, 4EBP1, S6RP, p70S6, NF $\kappa$ B and STAT3 was observed after treatment with all of the TKIs tested, with exception of 4EBP1 and S6RP after pazopanib treatment. We also found that, among the TKIs, pazopanib treatment lead less phosphorylation of NF $\kappa$ B, STAT3 and mTOR as well as HIF expression compared with sunitinib and sorafenib treatment. This might contribute that the safety and quality-of-life profiles favor pazopanib compared with sunitinib<sup>58</sup>). Treatment with the IL-6R blocking tocilizumab abolished the effect of the TKIs on the activation of those molecules, arguing that the observed TKI effects depend on the enhanced IL-6 signaling in 786-O cells. To study the impact of IL-6 on cell proliferation, we determined the effect of TKIs on 786-O cells under the blockade of IL-6R signaling by tocilizumab in a MTT assay. As a result, the addition of IL-6R antibody tocilizumab to TKIs results in a significant reduction of the 786-O cell count accompanied with decreased VEGF secretion. To confirm that tocilizumab improves the effect of low dose TKIs in the MTT assay, we employed a nude mice xenograft model. Consequently, the growth of tumors in athymic mice receiving combination therapy with tocilizumab and TKI was retarded in comparison with those in the tocilizumab, TKI and non-treated (PBS) groups. The vascularization of the xenograft tumors was also dependent on the treatment. Tumors of mice treated with TKI or tocilizumab alone showed a slightly reduced vascularization of the tumor, determined by CD31 staining. On the other hand, a remarkable

reduction of angiogenesis was observed when mice were treated with tocilizumab in combination with TKI. To confirm the possibility of prevention of TKI-resistance by combination therapy with TKI and tocilizumab *in vivo*, we employed FDG-PET imaging and evaluated during a time course of 3 and 21 days of challenge in nude mice xenograft model of 786-O cells. We found that SUVmax was significantly decreased in the tumor in the combination therapy compared with TKI alone at day 3 and it remained low at day 21 which means decreased tumor viability. Immunohistochemical examinations revealed that decreased CD31 positive cells at day 3, however, the CD31 positive cells increased again in the tumor region treated with TKI alone on the day 21 which would indicate the TKI-resistance. In contrast, when TKI was given in combination with tocilizumab, the tumors showed extensive central necrosis with absence of CD31 positive cells. Our findings suggest that a combination therapy using an antihuman IL-6R antibody with TKIs may represent a novel therapeutic approach for the antiangiogenic treatment of RCC<sup>59</sup>). In addition, heparan sulfate-specific endosulfatase-2 (SULF-2) can modulate the signaling of heparan sulfate proteoglycan-binding proteins like VEGF. SULF-2 negatively regulates VEGF in several cell lines through modification of heparan sulfate proteoglycan (HSPG)<sup>60</sup>). Among the RCC cell lines, 786-O showed a lower level of SULF-2 expression compared with ACHN cells. The RCC with low SULF-2 expression might have a higher potential for cell invasion and proliferation leading to a poorer prognosis for the RCC patients<sup>61</sup>). It would be interesting to investigate the role of SULF-2 in TKI resistance. These experiments are underway.

## Conclusions

RCC cells secrete IL-6 by treatment with IFN as well as TKIs, and activates STAT3, consequently resulting in a secretion of IL-6 and VEGF which lead drug resistance (Figure). The clinical use of IL-6/JAK/STAT3-targeting agents will be beneficial in the treatment of certain cancers and IL-6/STAT3 inhibitors reached phase I/II clinical trials<sup>29</sup>). Inhibition of the IL-6 signaling by IL-6R antibody tocilizumab may re-activate the anti-tumor activity of anti RCC drugs *ie* IFN and TKIs. Clinical trial for the combination therapy with anti-RCC drugs and IL-6R antibody might be needed.

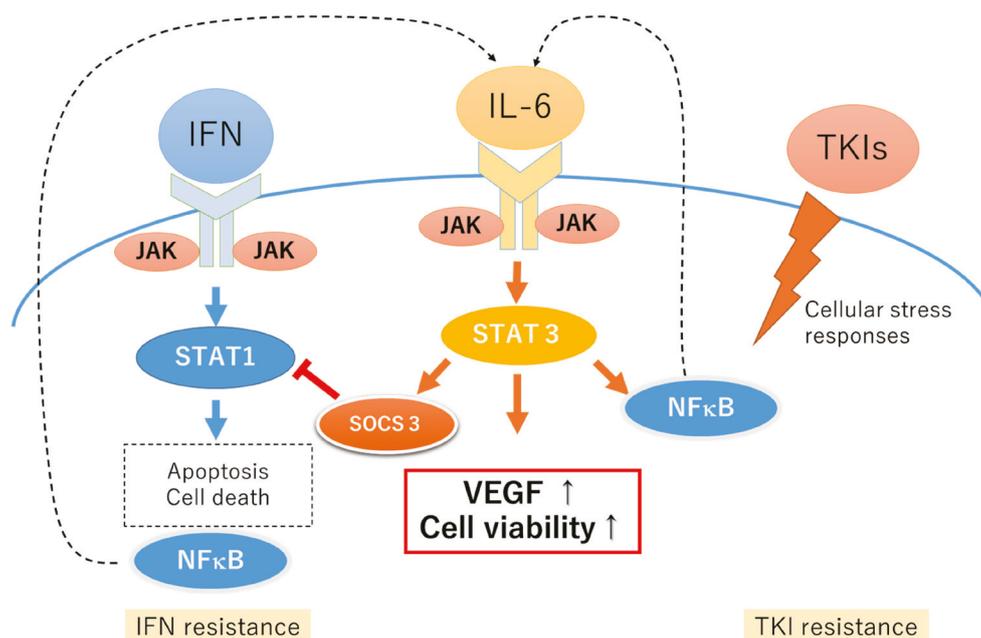


Fig. IL-6 mediated drug resistance in RCC cells. Following anti-RCC drug stimulation, IL-6 is secreted by RCC cells. It then binds IL-6 receptor and results in activation of the JAK/STAT3 signaling pathway, leading to the transcription of STAT3 target genes, *i.e.* VEGF or SOCS3. SOCS3 suppresses STAT1 activation. STAT3 and NF- $\kappa$ B interact at multiple levels, and promote inflammation, increasing tumor cell proliferation and survival as well as tumor angiogenesis and metastasis<sup>27</sup>. IL-6 can also activate the AKT/mTOR pathways.

## References

- Cohen HT, McGovern FJ. Renal-cell carcinoma. *N Engl J Med*, **353**(23): 2477-2490, 2005.
- Escudier B, Eisen T, Stadler WM, Szczylik C, Oudard S, Siebels M, *et al.* Sorafenib in advanced clear-cell renal-cell carcinoma. *N Engl J Med*, **356**(2): 125-134, 2007.
- Motzer RJ, Hutson TE, Tomczak P, Michaelson MD, Bukowski RM, Rixe O, *et al.* Sunitinib versus interferon alfa in metastatic renal-cell carcinoma. *N Engl J Med*, **356**(2): 115-124, 2007.
- Motzer RJ, Michaelson MD, Rosenberg J, Bukowski RM, Curti BD, George DJ, *et al.* Sunitinib efficacy against advanced renal cell carcinoma. *J Urol*, **178**(5): 1883-1887, 2007.
- Motzer RJ, Rini BI, McDermott DE, Redman BG, Kuzel TM, Harrison MR, *et al.* Nivolumab for Metastatic Renal Cell Carcinoma: Results of a Randomized Phase II Trial. *J Clin Oncol*, **33**(13): 1430-1437, 2015.
- Motzer RJ, Russo P. Systemic therapy for renal cell carcinoma. *J Urol*, **163**(2): 408-417, 2000.
- Vuky J, Motzer RJ. Cytokine therapy in renal cell cancer. *Urol Oncol*, **5**(6): 249-257, 2000.
- Interferon-alpha and survival in metastatic renal carcinoma: early results of a randomised controlled trial. Medical Research Council Renal Cancer Collaborators. *Lancet*, **353**(9146): 14-17, 1999.
- Pyrhonen S, Salminen E, Ruutu M, Lehtonen T, Nurmi M, Tammela T, *et al.* Prospective randomized trial of interferon alfa-2a plus vinblastine versus vinblastine alone in patients with advanced renal cell cancer. *J Clin Oncol*, **17**(9): 2859-2867, 1999.
- Akaza H, Tsukamoto T, Onishi T, Miki T, Kinouchi T, Naito S. A low-dose combination therapy of interleukin-2 and interferon-alpha is effective for lung metastasis of renal cell carcinoma: a multicenter open study. *Int J Clin Oncol*, **11**(6): 434-440, 2006.
- Akaza H, Kawai K, Tsukamoto T, Fujioka T, Tomita Y, Kitamura T, *et al.* Successful outcomes using combination therapy of interleukin-2 and interferon-alpha for renal cell carcinoma patients with lung metastasis. *Jpn J Clin Oncol*, **40**(7): 684-689, 2010.
- Naito S, Yamamoto N, Takayama T, Muramoto M, Shinohara N, Nishiyama K, *et al.* Prognosis of Japanese metastatic renal cell carcinoma patients in the cytokine era: a cooperative group report of 1463 patients. *Eur Urol*, **57**(2): 317-325, 2010.
- Motzer RJ, Hutson TE, Tomczak P, Michaelson MD, Bukowski RM, Oudard S, *et al.* Overall survival and updated results for sunitinib compared with interferon alfa in patients with metastatic renal cell carcinoma. *J Clin Oncol*, **27**(22): 3584-

- 3590, 2009.
14. Adnane L, Trail PA, Taylor I, Wilhelm SM. Sorafenib (BAY 43-9006, Nexavar), a dual-action inhibitor that targets RAF/MEK/ERK pathway in tumor cells and tyrosine kinases VEGFR/PDGFR in tumor vasculature. *Methods Enzymol*, **407** : 597-612, 2006.
  15. Motzer RJ, Michaelson MD, Redman BG, Hudes GR, Wilding G, Figlin RA, *et al.* Activity of SU11248, a multitargeted inhibitor of vascular endothelial growth factor receptor and platelet-derived growth factor receptor, in patients with metastatic renal cell carcinoma. *J Clin Oncol*, **24**(1) : 16-24, 2006.
  16. Gupta S, Spiess PE. The prospects of pazopanib in advanced renal cell carcinoma. *Ther Adv Urol*, **5**(5) : 223-232, 2013.
  17. Rixe O, Bukowski RM, Michaelson MD, Wilding G, Hudes GR, Bolte O, *et al.* Axitinib treatment in patients with cytokine-refractory metastatic renal-cell cancer : a phase II study. *Lancet Oncol*, **8**(11) : 975-984, 2007.
  18. Rini BI, Escudier B, Tomczak P, Kaprin A, Szczylik C, Hutson TE, *et al.* Comparative effectiveness of axitinib versus sorafenib in advanced renal cell carcinoma (AXIS) : a randomised phase 3 trial. *Lancet*, **378**(9807) : 1931-1939, 2011.
  19. Coppin C, Kollmannsberger C, Le L, Porzolt F, Wilt TJ. Targeted therapy for advanced renal cell cancer (RCC) : a Cochrane systematic review of published randomised trials. *BJU Int*, **108**(10) : 1556-1563, 2011.
  20. Rini BI, Atkins MB. Resistance to targeted therapy in renal-cell carcinoma. *Lancet Oncol*, **10**(10) : 992-1000, 2009.
  21. Del Bufalo D, Ciuffreda L, Trisciuglio D, Desideri M, Cognetti F, Zupi G, *et al.* Antiangiogenic potential of the Mammalian target of rapamycin inhibitor temsirolimus. *Cancer Res*, **66**(11) : 5549-5554, 2006.
  22. Bjornsti MA, Houghton PJ. The TOR pathway : a target for cancer therapy. *Nat Rev Cancer*, **4**(5) : 335-348, 2004.
  23. Motzer RJ, Escudier B, McDermott DF, George S, Hammers HJ, Srinivas S, *et al.* Nivolumab versus Everolimus in Advanced Renal-Cell Carcinoma. *N Engl J Med*, **373**(19) : 1803-1813, 2015.
  24. Kumari N, Dwarakanath BS, Das A, Bhatt AN. Role of interleukin-6 in cancer progression and therapeutic resistance. *Tumour Biol*, **37**(9) : 11553-11572, 2016.
  25. Bournazou E, Bromberg J. Targeting the tumor microenvironment : JAK-STAT3 signaling. *JAK-STAT*, **2**(2) : e23828, 2013.
  26. Yu H, Jove R. The STATs of cancer—new molecular targets come of age. *Nat Rev Cancer*, **4**(2) : 97-105, 2004.
  27. Yu H, Pardoll D, Jove R. STATs in cancer inflammation and immunity : a leading role for STAT3. *Nat Rev Cancer*, **9**(11) : 798-809, 2009.
  28. Heinrich PC, Behrmann I, Haan S, Hermanns HM, Muller-Newen G, Schaper F. Principles of interleukin (IL)-6-type cytokine signalling and its regulation. *Biochem J*, **374**(Pt 1) : 1-20, 2003.
  29. Johnson DE, O'Keefe RA, Grandis JR. Targeting the IL-6/JAK/STAT3 signalling axis in cancer. *Nat Rev Clin Oncol*, **15**(4) : 234-248, 2018.
  30. Dancey J. mTOR signaling and drug development in cancer. *Nat Rev Clin Oncol*, **7**(4) : 209-219, 2010.
  31. Hideshima T, Nakamura N, Chauhan D, Anderson KC. Biologic sequelae of interleukin-6 induced PI3-K/Akt signaling in multiple myeloma. *Oncogene*, **20**(42) : 5991-6000, 2001.
  32. Shi Y, Hsu JH, Hu L, Gera J, Lichtenstein A. Signal pathways involved in activation of p70S6K and phosphorylation of 4E-BP1 following exposure of multiple myeloma tumor cells to interleukin-6. *J Biol Chem*, **277**(18) : 15712-15720, 2002.
  33. Chang Q, Bournazou E, Sansone P, Berishaj M, Gao SP, Daly L, *et al.* The IL-6/JAK/Stat3 feed-forward loop drives tumorigenesis and metastasis. *Neoplasia*, **15**(7) : 848-862, 2013.
  34. Atsumi T, Singh R, Sabharwal L, Bando H, Meng J, Arima Y, *et al.* Inflammation amplifier, a new paradigm in cancer biology. *Cancer Res*, **74**(1) : 8-14, 2014.
  35. Ludwig H, Nachbaur DM, Fritz E, Krainer M, Huber H. Interleukin-6 is a prognostic factor in multiple myeloma. *Blood*, **77**(12) : 2794-2795, 1991.
  36. Kusaba T, Nakayama T, Yamazumi K, Yakata Y, Yoshizaki A, Inoue K, *et al.* Activation of STAT3 is a marker of poor prognosis in human colorectal cancer. *Oncol Rep*, **15**(6) : 1445-1451, 2006.
  37. Chen Y, Wang J, Wang X, Liu X, Li H, Lv Q, *et al.* STAT3, a Poor Survival Predictor, Is Associated with Lymph Node Metastasis from Breast Cancer. *J Breast Cancer*, **16**(1) : 40-49, 2013.
  38. Macha MA, Matta A, Kaur J, Chauhan SS, Thakar A, Shukla NK, *et al.* Prognostic significance of nuclear pSTAT3 in oral cancer. *Head Neck*, **33**(4) : 482-489, 2011.
  39. Negrier S, Perol D, Menetrier-Caux C, Escudier B, Pallardy M, Ravaud A, *et al.* Interleukin-6, interleukin-10, and vascular endothelial growth factor in metastatic renal cell carcinoma : prognostic value of interleukin-6—from the Groupe Francais d'Immunotherapie. *J Clin Oncol*, **22**(12) : 2371-2378, 2004.
  40. Montero AJ, Diaz-Montero CM, Millikan RE, Liu J, Do KA, Hodges S, *et al.* Cytokines and angiogenic factors in patients with metastatic renal cell carcinoma.

- noma treated with interferon- $\alpha$  : association of pretreatment serum levels with survival. *Ann Oncol*, **20**(10) : 1682-1687, 2009.
41. Ramsey S, Aitchison M, Graham J, McMillan DC. The longitudinal relationship between the systemic inflammatory response, circulating T-lymphocytes, interleukin-6 and -10 in patients undergoing immunotherapy for metastatic renal cancer. *BJU Int*, **102**(1) : 125-129, 2008.
  42. Yoshimura A, Naka T, Kubo M. SOCS proteins, cytokine signalling and immune regulation. *Nat Rev Immunol*, **7**(6) : 454-465, 2007.
  43. Hashimoto K, Ishibashi K, Ishioka K, Zhao D, Sato M, Ohara S, *et al.* RSV replication is attenuated by counteracting expression of the suppressor of cytokine signaling (SOCS) molecules. *Virology*, **391**(2) : 162-170, 2009.
  44. Takahashi Y, Kaneda H, Takasuka N, Hattori K, Nishikawa M, Watanabe Y, *et al.* Enhancement of antiproliferative activity of interferons by RNA interference-mediated silencing of SOCS gene expression in tumor cells. *Cancer Sci*, **99**(8) : 1650-1655, 2008.
  45. Song MM, Shuai K. The suppressor of cytokine signaling (SOCS) 1 and SOCS3 but not SOCS2 proteins inhibit interferon-mediated antiviral and antiproliferative activities. *J Biol Chem*, **273**(52) : 35056-35062, 1998.
  46. Tomita S, Ishibashi K, Hashimoto K, Sugino T, Yanagida T, Kushida N, *et al.* Suppression of SOCS3 increases susceptibility of renal cell carcinoma to interferon- $\alpha$ . *Cancer Sci*, **102**(1) : 57-63, 2011.
  47. Nemeth E, Valore EV, Territo M, Schiller G, Lichtenstein A, Ganz T. Hepcidin, a putative mediator of anemia of inflammation, is a type II acute-phase protein. *Blood*, **101**(7) : 2461-2463, 2003.
  48. Motzer RJ, Bacik J, Murphy BA, Russo P, Mazumdar M. Interferon- $\alpha$  as a comparative treatment for clinical trials of new therapies against advanced renal cell carcinoma. *J Clin Oncol*, **20**(1) : 289-296, 2002.
  49. Starr R, Willson TA, Viney EM, Murray LJ, Rayner JR, Jenkins BJ, *et al.* A family of cytokine-inducible inhibitors of signalling. *Nature*, **387**(6636) : 917-921, 1997.
  50. Nishimoto N, Kishimoto T. Inhibition of IL-6 for the treatment of inflammatory diseases. *Curr Opin Pharmacol*, **4**(4) : 386-391, 2004.
  51. Oguro T, Ishibashi K, Sugino T, Hashimoto K, Tomita S, Takahashi N, *et al.* Humanised antihuman IL-6R antibody with interferon inhibits renal cell carcinoma cell growth in vitro and in vivo through suppressed SOCS3 expression. *Eur J Cancer*, **49**(7) : 1715-1724, 2013.
  52. Powles T, Staehler M, Ljungberg B, Bensalah K, Canfield SE, Dabestani S, *et al.* Updated EAU Guidelines for Clear Cell Renal Cancer Patients Who Fail VEGF Targeted Therapy. *Eur Urol*, **69**(1) : 4-6, 2016.
  53. Huang WC, Hung CM, Wei CT, Chen TM, Chien PH, Pan HL, *et al.* Interleukin-6 expression contributes to lapatinib resistance through maintenance of stemness property in HER2-positive breast cancer cells. *Oncotarget*, **7**(38) : 62352-62363, 2016.
  54. Xu Z, Yang F, Wei D, Liu B, Chen C, Bao Y, *et al.* Long noncoding RNA-SRLR elicits intrinsic sorafenib resistance via evoking IL-6/STAT3 axis in renal cell carcinoma. *Oncogene*, **36**(14) : 1965-1977, 2017.
  55. Mu X, Zhao T, Xu C, Shi W, Geng B, Shen J, *et al.* Oncometabolite succinate promotes angiogenesis by upregulating VEGF expression through GPR91-mediated STAT3 and ERK activation. *Oncotarget*, **8**(8) : 13174-13185, 2017.
  56. Zhao D, Pan C, Sun J, Gilbert C, Drews-Elger K, Azzam DJ, *et al.* VEGF drives cancer-initiating stem cells through VEGFR-2/Stat3 signaling to upregulate Myc and Sox2. *Oncogene*, **34**(24) : 3107-3119, 2015.
  57. Dodd KM, Yang J, Shen MH, Sampson JR, Tee AR. mTORC1 drives HIF-1 $\alpha$  and VEGF-A signaling via multiple mechanisms involving 4E-BP1, S6K1 and STAT3. *Oncogene*, **34**(17) : 2239-2250, 2015.
  58. Motzer RJ, Hutson TE, Cella D, Reeves J, Hawkins R, Guo J, *et al.* Pazopanib versus sunitinib in metastatic renal-cell carcinoma. *N Engl J Med*, **369**(8) : 722-731, 2013.
  59. Ishibashi K, Haber T, Breuksch I, Gebhard S, Sugino T, Kubo H, *et al.* Overriding TKI resistance of renal cell carcinoma by combination therapy with IL-6 receptor blockade. *Oncotarget*, **8**(33) : 55230-55245, 2017.
  60. Mitsi M, Hong Z, Costello CE, Nugent MA. Heparin-mediated conformational changes in fibronectin expose vascular endothelial growth factor binding sites. *Biochemistry*, **45**(34) : 10319-10328, 2006.
  61. Kumagai S, Ishibashi K, Kataoka M, Oguro T, Kiko Y, Yanagida T, *et al.* Impact of Sulfatase-2 on cancer progression and prognosis in patients with renal cell carcinoma. *Cancer Sci*, **107**(11) : 1632-1641, 2016.