Original paper

The role of interleukin-6 classic and trans-signaling and interleukin-11 classic signaling in gastric cancer cells

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Introduction: The cytokine interleukin-11 (IL-11) binds on its target cells to a membrane-bound IL-11R, which results in homodimerization of the signal-transducing β-receptor gp130. Recent studies have uncovered a pro-inflammatory role in several diseases, including different tumor entities, and mouse models have revealed a crucial role of the IL-11/IL-11R axis in gastric cancer. However, studies regarding human gastric cancer are sparse, and whether the results obtained in mouse models also hold true in the human situation is little investigated.

Material and methods: We analyzed gene expression of *IL11RA*, *IL11*, *IL6R*, *IL6* and *IL6ST* in different gastric tumor and immune cell lines. Furthermore, we stimulated these cell lines with exogenous cytokines and determined intracellular signaling. Finally, we analyzed gene expression data of gastric tumor patients and correlated them with overall patient survival.

Results: This study showed that different gastric tumor cell lines respond to IL-6 classic and trans-signaling, but only slightly to stimulation with IL-11. We observed that monocyte-like cell lines expressed high levels of IL-6R and responded to IL-6, but not to stimulation with IL-11. Using gene expression data, we found that *IL11RA* and *IL11* are not overexpressed in human gastric cancer tissue and do not correlate with patient survival. However, low *IL6* expression is associated with higher overall survival.

Conclusions: We provided evidence for IL-6 but not IL-11 signaling in gastric tumor cells and demonstrated that *IL6* expression in gastric tumors is associated with overall survival of patients.

Key words: interleukin-6, interleukin-6 receptor, interleukin-11, interleukin-11 receptor, gp130, gastric cancer.

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Introduction

Gastric cancer is the cancer type with the fifth highest incidence and fourth highest mortality, resulting in more than 1 million new cases and more than 750,000 deaths every year [1]. In 2020, there were more than 5,000 new cases in female patients and more than 9,000 new cases in male patients in Germany [2]. The relative five-year survival rate is only 37% in women and 35% in men [2]. Risk factors to develop a gastric tumor are manifold and include, among others, *Helicobacter pylori* infection, gastroesophageal reflux, high salt intake, being male, and consuming alcohol and red meat [3, 4]. Both mortality and morbidity of gastric cancer are declining, probably due to better hygiene, a reduction in *Helicobacter pylori* infections and improvements in terms of therapies [5, 6]. Current treatment options include endoscopic resection, surgery with lymphadenectomy and different chemotherapy regimens [7]. Gastric tumors are infiltrated with immune cells, which can be used not only to identify different molecular subtypes, but also to predict the response to immune checkpoint inhibitors [8].

Interleukin-6 (IL-6) and IL-11 both belong to the IL-6 family of cytokines [9] and have partly overlapping, but mostly unique, biological functions [10]. Both cytokines can be secreted by a variety of different cell types, including different types of immune cells, epithelial and endothelial cells and fibroblasts [10, 11]. A typical inducer of IL-6 production is lipopolysaccharide derived from gram-negative bacteria [12], while IL-11 is induced e.g. by transforming growth factor (TGF-β1) or infection with respiratory syncytial virus [13]. IL-6 and IL-11 activate their target cells *via* binding to non-signaling α-receptors at the cell surface (IL-6R and IL-11R, respectively). The resulting IL-6/IL-6R and IL-11/IL-11R complexes induce the formation of a homodimer of the signal-transducing β-receptor gp130, which in turn activates intracellular signaling cascades, most notably the Janus kinase/signal transducer and activator of transcription (Jak/STAT) pathway [9, 14]. Here, Jak1 phosphorylates tyrosine residues within the intracellular part of gp130, which serve as docking sites for STAT3, which are then also phosphorylated and translocate into the nucleus to induce target gene transcription [14, 15]. While gp130 is ubiquitously expressed, IL-6R and IL-11R expression are restricted to certain cell types, and thus the expression pattern of IL-6R and IL-11R dictates which cell type responds to which cytokine. However, soluble forms of both IL-6R and IL-11R (sIL-6R/sIL-11R) exist, which are generated by proteolytic cleavage of the membrane-bound receptors and are able to bind their ligand with the same affinity [16–20]. Signaling *via* soluble receptors is called trans-signaling and accounts in the case of IL-6 for the pro-inflammatory properties of the cytokine, while this is not known for IL-11 yet [21–23].

Serum levels of both IL-6 and IL-11 are very low or even undetectable in healthy humans, but can rise substantially during disease, which is exem-

plified by IL-6 levels of more than 100 ng/ml in patients with meningococcal septic shock [24]. In most inflammatory diseases, however, serum levels of IL-6 and IL-11 are in the pg/ml or low ng/ml range. The involvement of IL-6 in most inflammatory diseases made the cytokine and its receptor an attractive therapeutic target, which resulted in the development and clinical admission of several antibodies blocking either IL-6 or the IL-6R [25, 26]. Selective blockade of IL-6 trans-signaling, which appears to be superior compared to global blockade of IL-6 [27], is currently being evaluated in patients with inflammatory bowel disease [28, 29] and atherosclerotic cardiovascular disease [30]. Antibodies targeting IL-11 or the IL-11R are currently under development, as IL-11 appears to be a druggable target in different fibrotic diseases [31, 32].

Chronic inflammation is closely correlated to gastric tumorigenesis, and cytokines of the IL-6 family play crucial roles in both processes [33, 34]. *Helicobacter pylori* infection increases IL-6 expression in patients and both IL-6 and IL-11 have been reported to be strongly up-regulated in biopsies of gastric cancer patients [35]. IL-11 has also been identified by proteomics in human gastric tumor samples [36]. In addition, several mechanistic studies have investigated the roles of IL-6, IL-11, gp130 and STAT3 using rodent models with genetically modified mice. The $gp130^{757F/F}$-mice, in which the tyrosine residue 757 of gp130 is replaced by a phenylalanine residue, resulting in loss of negative feedback and thus overactivation of gp130-mediated STAT3 signaling, develops gastric tumors spontaneously [37]. The major driver in this model is IL-11, as genetic deletion of the IL-11R prevents gastric tumor formation, while deletion of IL-6 has little to no impact [38–41].

In the present study, we analyzed expression of IL-6R, IL-11R and gp130 in three gastric tumor cell lines and three monocytic immune cell lines. Using recombinant proteins, we analyzed the response of the different cell lines to stimulation with the different cytokines. Furthermore, we used *in silico* analyses to evaluate the prognostic value of the expression of the different genes in terms of overall survival of gastric cancer patients.

Material and methods

In silico analyses

All *in silico* analyses were performed using GEPIA2 (http://gepia2.cancer-pku.cn/) [42]. Data were retrieved and analyses performed on December 6th, 2023. For this study, we used the stomach adenocarcinoma (STAD) dataset, consisting of 408 tumor samples, 36 normal controls, and 175 GTEx samples (stomach). We generated survival plots using the "survival analysis" package using standard parameters except that we did not include the 95% confidence interval. We used the "Expression DIY" package to generate the box plots for the expression analysis of the different genes in tumors compared to normal and GTEx data using standard parameters.

Reagents

The following primary antibodies were used: β-actin (D6A8), GAPDH (D16H11) XP® Rabbit mAb, phospho-STAT3 (Tyr705, D3A7) XP® Rabbit mAb and STAT3 (124H6) Mouse mAb (all from Cell Signaling Technology, Danvers, MA, USA). Secondary antibodies were anti-mouse IgG, HRP-linked antibody and anti-rabbit IgG, HRP-linked antibody (both also from Cell Signaling Technology). Hyper-IL-6, IL-6 and IL-11 were produced in house as described previously [43–45].

Cell lines and cell culture

CLS-103, NCI-N87, THP-1 and U-937 cells were cultivated in RPMI 1640 medium (PAN-Biotech or Thermo Fisher Scientific). J774A.1 cells were cultured in DMEM medium and AGS cells in Ham's medium (both from PAN-Biotech, Aidenbach, Germany). All media were supplemented with 10% fetal bovine serum (Thermo Fisher Scientific, Waltham, USA), 100 U/ml penicillin and 100 µg/ml streptomycin (PAN-Biotech, Aidenbach, Germany). Ham's medium was additionally supplemented with 200 mM L-glutamine (PAN-Biotech, Aidenbach, Germany). All cells were kept at 37°C and 5% CO_2 in a standard incubator with a water-saturated atmosphere.

Stimulation of cell lines with cytokines

In order to analyze STAT3 signaling in the different cell lines, the following cell numbers of adherent cells were seeded in 6-well plates in 2 ml medium *per* well: 5×10^5 AGS cells, 5×10^5 CLS-103 cells and 1×10^6 NCI-N87 cells. The cells were placed in the incubator overnight and the medium was removed the next day. After three washing steps with 1 ml PBS each, the cells were serum-starved for 24 hours in 1 ml of medium without serum. The suspension cell lines were collected by centrifugation, the cells washed three times with 10 ml of PBS each, and then re-suspended in serum-free medium. The following cell numbers were seeded in 12-well plates in 1 ml of medium *per* well: 3×10^6 J774.A1 cells, 1.5×10^6 THP-1 cells and 1.5×10^6 U-937 cells. The monocyte cell lines were serum starved for 6 hours. After incubation, cytokines were added to the medium at the following concentrations: 10 ng/ml IL-6, 10 ng/ml IL-11 and 10 ng/ml Hyper-IL-6 (HyIL-6). For THP-1 and U-937 cells, 50 ng/ml Hyper-IL-6 was used instead. The cells were placed back into the incubator for 15 min. The cells were detached and washed in PBS and finally lysed in lysis buffer (10 mM Tris-HCl, 140 mM NaCl, 5 mM EDTA, 1% Triton-X 100, protease inhibitor cocktail and phosphatase inhibitor cocktail (both Sigma-Aldrich), pH 7.5).

Western blotting

Cell lysates were separated by SDS-PAGE and transferred to nitrocellulose membranes. Membranes were blocked in tris-buffered saline (TBS) supplemented with 5% albumin fraction V and 0.2% Nonidet P40 for 1 h at room temperature. Afterwards, the membranes were incubated with the primary antibodies (diluted 1 : 1,000 in TBS-T [TBS supplemented with 0.25% Tween-20] with 5% bovine serum albumin) at 4°C overnight. After washing three times with TBS-T, the membranes were incubated with secondary antibodies (diluted 1 : 3,000 in TBS-T supplemented with 5% milk powder) for 1 hour at room

temperature. Membranes were again washed with TBS-T and afterwards incubated with Immobilon Western HRP substrate (Millipore by Merck, Darmstadt, Germany) and detected using the Western Blot Imager FluorChem E (Biosciences, Santa Clara, USA). For detection of total STAT3 and the loading control GAPDH after detection of pSTAT3, membranes were stripped, washed with TBS-T, and again blocked and incubated with antibodies as described above.

Densitometric analysis

To quantify pSTAT3 levels, western blot images were densitometrically analyzed using the Image Studio Lite software (LI-COR Biosciences) and the ratio of the pSTAT3/STAT3 signal intensities was calculated in relation to the unstimulated control.

RNA isolation and quantitative real time polymerase chain reaction

RNA was isolated using the NucleoSpin RNA kit (Macherey-Nagel) according to the manufacturer's instructions. RNA was transcribed into cDNA using an oligo-dT-primer and Revert Aid Reverse Transcriptase (Thermo Fisher Scientific, Waltham, USA). Quantitative real time polymerase chain reaction (qRT-PCR) was performed using the des FAST SYBR Green Master Mix (Thermo Fisher Scientific, Waltham, USA) and the following oligonucleotides: hGAPDH (forward: 5′ AGCCACATCGCTCAGACAC 3′; reverse: 5′ GCCCAATACGACCAAATCC 3′), hIL6ST (forward: 5′ GGGCAATATGACTCTTTGAAGG 3′; reverse: 5′ TTCCTGTTGATGTTCAGAATGG 3′), hIL6 (forward: 5′ CACCAGGCAAGTCTCCTCAT 3′; reverse: 5′ TCCAAAGATGTAGCCGCCCC 3′), hIL6RA (forward: 5′ GCTGAACTTGCTCCCGACAC 3′; reverse: 5′ AAGAGCCCCCTCAGCAATGT 3′), hIL11 (forward: 5′ GGACAGGGAAGGGTTAAAGG 3′; reverse: 5′ CTCAGCACGACCAGGACC 3′), hIL11RA (forward: 5′ GGCTATGGACACTCTGCTGC 3′; reverse: 5′ GCAGACACCAGGGCTGTAG 3′), mGAPDH (forward: 5′ AAGGTCATCCCAGAGCTGAA 3′; reverse: 5′ CTGCTTCACCACCTTCTTGA 3′), mIL6ST (forward: 5′ ACCTGCTCTGGGTGGAATGG 3′; reverse: 5′CGGCTTGTTTGAGGTACGCC 3′), mIL6 (forward: 5′ TCCAGTTGCCTTCTTGGGAC 3′; reverse: 5′ GTGTAATTAAGCCTCCGACTTG 3′), mIL6RA (forward 5′ CATCTGTGAGTGGCGTCCGA 3′; reverse: 5′ TCGTTGTGGCTGGACTTGCT 3′), mIL11 (forward: 5′ CTGCACAGATGAGAGACAAATTCC 3′; reverse: 5′ GAAGCTGCAAAGATCCCAATG 3′) and mIL11RA (forward: 5′AATACCGACCAGCACAGCAT 3′; reverse: 5′ TGACTCGTACCGCGTGTG 3′).

Data were analyzed using the QuantStudio 6 Flex RT-PCR System (Thermo Fisher Scientific, Waltham, USA). The $2^{-\Delta CT}$ method was used, and gene expression was determined relative to the GAPDH amount of the cells.

Statistical analysis

Data were analyzed by one-way ANOVA following Dunnett's multiple comparison test. Data analyses were performed using GraphPad Prism version 10 (GraphPad Software, La Jolla California USA, www.graphpad.com). We performed a comparison of untreated cells with all treated conditions. Statistical significance was determined at a level of $p < 0.05$ and the p-values are shown in the respective diagrams of the figures. In addition, the statistical test used is described in the respective figure legends.

Results

Gastric cancer cell lines express only small amounts of IL-11R and IL-11

IL-6 binds to the IL-6R and IL-11 binds to the IL-11R. Both cytokines engage a homodimer of the signal-transducing β-receptor gp130 to activate intracellular signaling cascades in their respective target cells [10]. In order to analyze which cytokines might play a role in gastric tumors, we first analyzed expression of genes encoding the five proteins of interest in three different gastric cancer cell lines using qRT-PCR. In AGS cells, which is a cell line from a female patient with gastric adenocarcinoma, we could detect mRNA for *IL6* (expression relative to GAPDH 0.05 ±0.02) and *IL6R* (relative expression 0.1 ±0.02) (Fig. 1 A). In contrast, the expression level of *IL11RA* was lower (relative expression 0.02 ±0.01), and *IL11* itself was barely detectable. Due to the ubiquitous expression of gp130, it was expected that *IL6ST* could be robustly detected (relative expression 1.34 ±0.41) (Fig. 1 A). In order

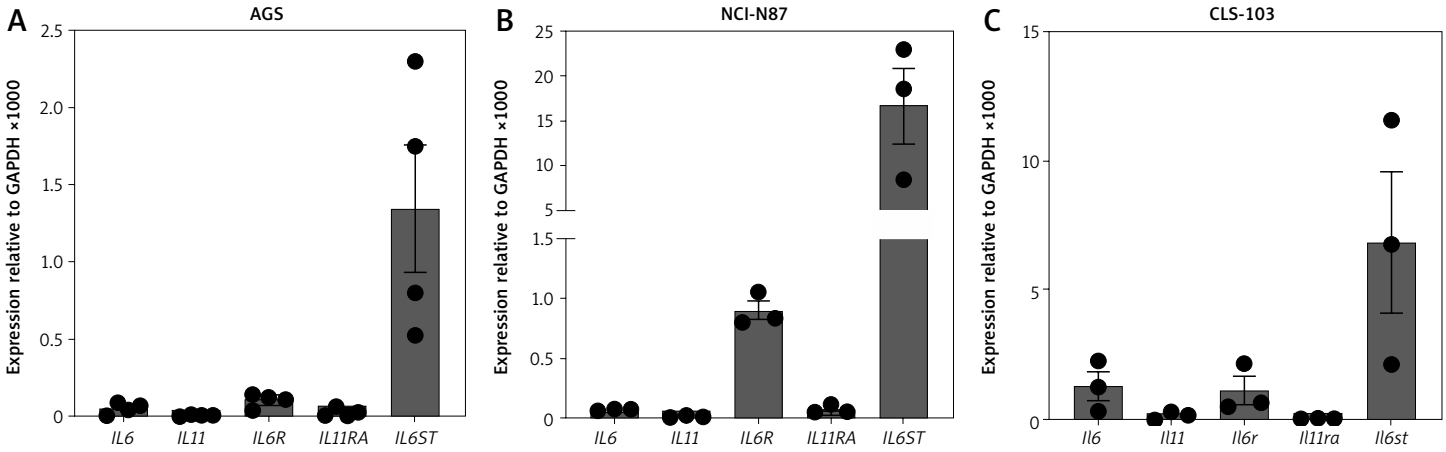

Fig. 1. High levels of *IL6ST* in gastric tumor cell lines. **A, B**) Expression of *IL6*, *IL11*, *IL6R*, *IL11RA* and *IL6ST* was analyzed via quantitative real time polymerase chain reaction (qRT-PCR) in AGS cells (**A**) and N87 cells (**B**). Data are shown as mean ± SD relative to GAPDH expression and are derived from three independent experiments. **C**) Expression of *Il6*, *Il11*, *Il6r*, *Il11ra* and *Il6st* was analyzed *via* qRT-PCR in CLS-103 cells Data are shown as mean ± SD relative to GAPDH expression and are derived from three or four independent experiments.

not to rely on a single cell line, we performed the same analysis with NCI-N87 cells, a cell line that was isolated from the stomach of a male patient with gastric carcinoma. We could again clearly detect *IL6ST* (relative expression 16.62 ±4.27) and *IL6R* expression (relative expression 0.9 ±0.08) (Fig. 1 B). However, expression of *IL6*, *IL11* and *IL11RA* was barely detectable (Fig. 1 B).

Because several of the studies regarding a functional role of IL-11 in gastric tumors have been performed in mice, we additionally did the same analysis with CLS-103 cells, a murine gastric squamous cell carcinoma cell line. As shown in Figure 1 C, CLS-103 cells expressed significant amounts of *Il6* (relative expression 1.25 ±0.56), *Il6ra* (relative expression 1.09 ±0.54) and *Il6st* (relative expression 6.8 ±2.7), but only a small amount of *Il11*, while *Il11ra* was not detectable. We concluded from these experiments that high IL-11R expression is not a feature of these gastric cancer cell lines, and that this is species-independent. In contrast, the consistent expression of the IL-6R suggests that IL-6 rather than IL-11 signaling might be crucially involved in gastric tumorigenesis.

Gastric cancer cell lines respond poorly to stimulation with exogenous IL-11

Having shown that gastric cancer cell lines express little *IL11R*, but much more *IL6R* mRNA, we next sought to validate whether they could be activated by stimulation with exogenous IL-6 or IL-11. We performed these experiments because mRNA levels do not always accurately reflect the actual protein level, and we have shown previously that rather low levels of IL-11R are sufficient to activate intracellular signaling cascades when cells are stimulated with IL-11 [46, 47]. For this, we seeded AGS cells, serum-starved them for 24 hours and either stimulated them for 15 min with 10 ng/ml IL-6, 10 ng/ml IL-11, or 10 ng/ml Hyper-IL-6, or left them untreated. Hyper-IL-6 is a fusion protein of IL-6 and the soluble IL-6R which can directly activate a gp130 homodimer without the need of a membrane-bound IL-6R and serves as the positive control [43]. Afterwards, the cells were lysed and the phosphorylation of the transcription factor STAT3 was analyzed by western blot. As shown in Figure 2 A, AGS cells responded weakly to stimulation with IL-6 and IL-11 (2.4 ±1-fold and 1.6 ±0.3-fold increase compared to unstimulated cells, respectively), while phosphorylation of STAT3 was significant when the cells were stimulated with Hyper-IL-6 (6.5 ±3.8-fold increase, $p = 0.0261$) (Fig. 2 A). NCI-N87 cells responded well to IL-6 stimulation (20.8 ±9.7-fold increase compared to the unstimulated control), which was not the case for IL-11 stimulation (3.7 ±1-fold increase) (Fig. 2 B). We observed again the strongest STAT3 activation when cells were stimulated with Hyper-IL-6 (120.7 ±113.4-fold increase) (Fig. 2 B). We obtained similar results with the CLS-103 cell line, which also responded to IL-6 (3.4 ±1.1-fold increase compared to the unstimulated control) and Hyper-IL-6 (29 ±8.8-fold increase, $p = 0.0002$) stimulation, but not when stimulated with IL-11 (1.1 ±0.1-fold increase) (Fig. 2 C). We concluded from these experiments that the analyzed gastric tumor cell lines express functional IL-6R at levels that make them responsive to IL-6 stimulation, but that the IL-11R levels are too low to provoke a meaningful biological response when stimulated with IL-11.

Immune cell lines express high levels of IL-6R

Immune cells play important yet not completely understood roles in gastric tumorigenesis [8, 48]. Because gastric tumor cell lines appear not to be the major targets of IL-11, we sought to elucidate whether immune cell lines might be targets of IL-11. For this, we first analyzed mRNA levels in THP-1 cells, a monocytic cell line isolated from a patient with acute monocytic leukemia, and U-937 cells, a pro-monocytic cell line isolated from a patient with histiocytic lymphoma. As shown in Figure 3 A and B, *IL6R* transcripts were highly abundant in these cell lines (relative expression 29.26 ±13.08 and 127.8 ±34.98 respectively), whereas *IL6ST* was detectable, but at lower levels (relative expression 0.64 ±0.09 and 2.74 ±0.73 respectively). *IL6*, *IL11* and *IL11R* could not be detected. In order to again not only rely on human cell lines, we performed the same analysis with J774A.1 cells, a murine monocyte-macrophage cell line. These cells expressed high levels of *IL6* (relative expression 0.7 ±0.5), *Il6ra* (relative expression 1.72 ±0.4) and *Il6st* (relative expression 2.33 ±0.63), but no *Il11* and *Il11ra* (Fig. 3 C). We concluded that at least these three immune cell-derived cell lines are also not primary target cells of IL-11, but nevertheless wanted to verify this at the functional level. As done with the gastric tumor cell lines before (Fig. 2), we serum-starved the cells for 5–6 hours, stimulated them with the indicated cytokines for 15 min, lysed the cells and determined STAT3 phosphorylation as the readout. THP-1 cells responded to stimulation with IL-6 (143.9 ±65.5-fold increase, $p = 0.0022$) and Hyper-IL-6 (49.5 ±16.8-fold increase) by phosphorylation of STAT3, but did not respond to IL-11 stimulation (Fig. 3 D). Likewise, U-937 cells showed strong phosphorylation of STAT3 in response to IL-6 (77.2 ±17-fold increase, $p = 0.0003$) and Hyper-IL-6 (29.3 ±20.1-fold increase), but not in response to IL-11 stimulation (Fig. 3 E). This was again not restricted to human cell lines, as J774A.1 cells also responded to IL-6 (19.2 ±12.9-fold increase) and Hyper-IL-6 treatment (53.1 ±1 3.5-fold increase, $p = 0.0004$), but not to IL-11 stimulation (Fig. 3 F). We concluded that also in immune cell lines IL-6, and not IL-11, is the dominant cytokine that is able to activate the cells.

Expression of *IL6*, but not *IL11* or *IL11R*, is predictive of overall survival in stomach adenocarcinoma patients

Our *in vitro* data so far suggest that IL-6 rather than IL-11 could potentially play a role in gastric tumorigenesis. In order to obtain further insight, we analyzed expression data from gastric cancer patients and controls using gene expression profiling interactive analysis 2 (GEPIA2) [42]. We analyzed the stomach adenocarcinoma (STAD) dataset, consisting of 408 tumor samples and 211 tissue-matched control samples. Interestingly, the tumor samples had significantly reduced amounts of *IL11RA* compared to the control tissue (Fig. 4 A). Amounts of *IL11* were increased in the tumor tissue compared to the controls, but this did not reach statistical significance (Fig. 4 B). Afterwards, we used survival data and divided the patients into

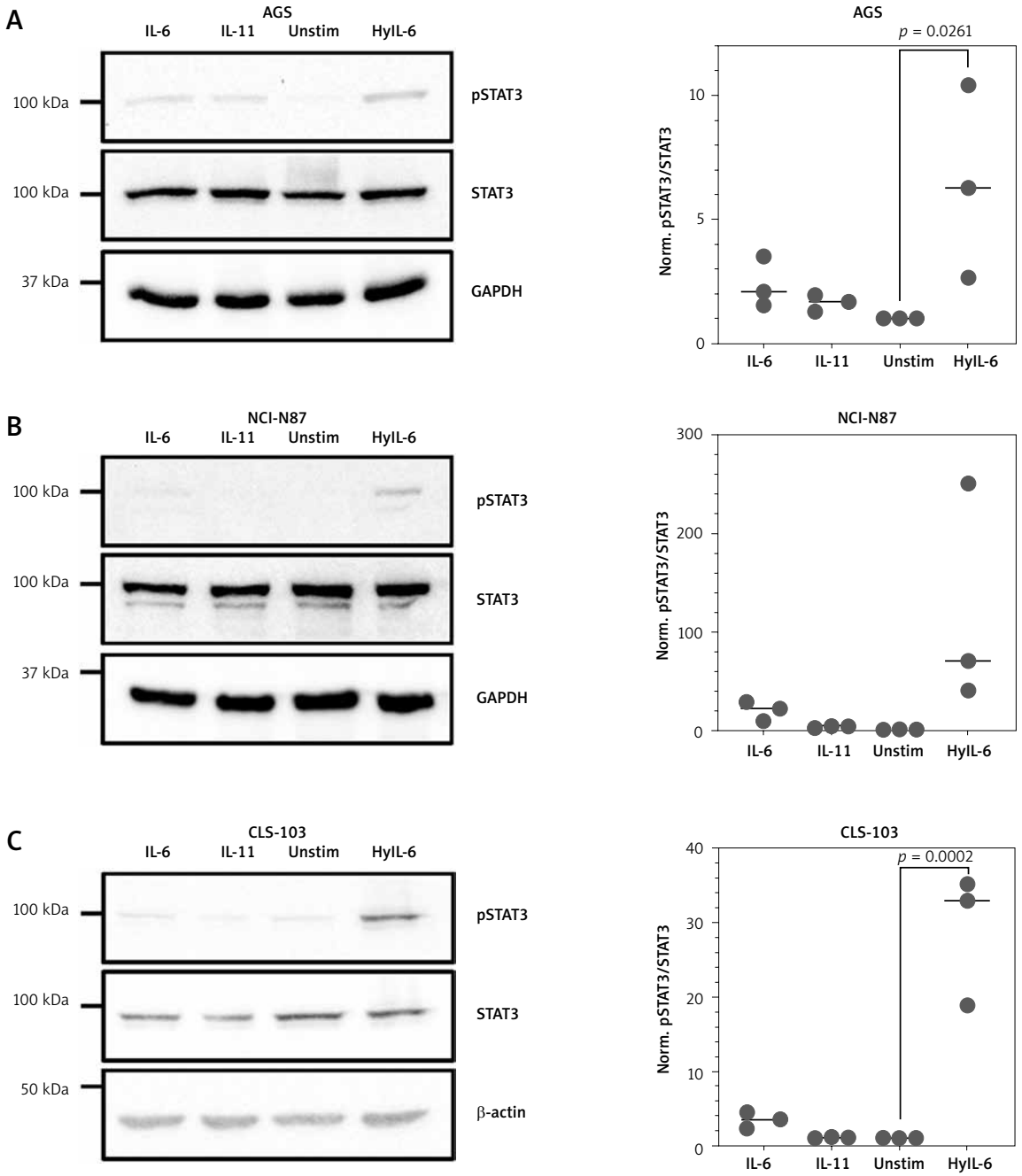

Fig. 2. Gastric cancer cell lines respond to IL-6 trans-signaling. Serum-starved AGS cells (**A**), N87 cells (**B**) and CLS-103 cells (**C**) were either stimulated with IL-6, IL-11, or Hyper-IL-6 for 15 min or left unstimulated. Afterwards, cells were lysed and phosphorylation of the transcription factor STAT3 was analyzed by western blot. STAT3 and GAPDH were analyzed to verify equal loading. One representative experiment is shown, and the corresponding densitometric analysis of the pSTAT3/STAT3 ratio (mean ± SD, n = 3) is shown next to the western blots. Statistical analysis was performed using one-way ANOVA following Dunnett's multiple comparison test. Here, all stimulated conditions were compared to the unstimulated cells and in case of statistical significance, the p value is shown above the respective bracket. All other comparisons were not significantly different

a low and a high expression group individually for both genes, resulting in 192 patients in each group (Fig. 4 C, D). In line with our previous findings, neither *IL11RA* ($p = 0.31$) (Fig. 4 C) nor *IL11* expression ($p = 0.66$) (Fig. 4 D) had a significant influence on the overall survival of the patients. Afterwards, we analyzed expression of *IL6R* and *IL6* in the same patient cohorts, which did not reveal any significant difference (Fig. 4 E, F). When we assessed overall survival by stratifying the patients again into a high and a low expressing group for both genes, we observed no difference when grouped according to *IL6R* expression (Fig. 4 G). However, the low *IL6* group had a significantly better overall survival compared to the high *IL6* group ($p = 0.01$) (Fig. 4 H). In summary, our expression analysis revealed no role for IL-11/IL-11R in overall survival of stomach adenocarcinoma patients, but showed that low levels of IL-6 were associated with better overall survival of these patients.

Discussion

Gastric cancer represents a major challenge to treat and therefore results in around one million deaths every year [1]. Although the number of affected patients has been decreas-

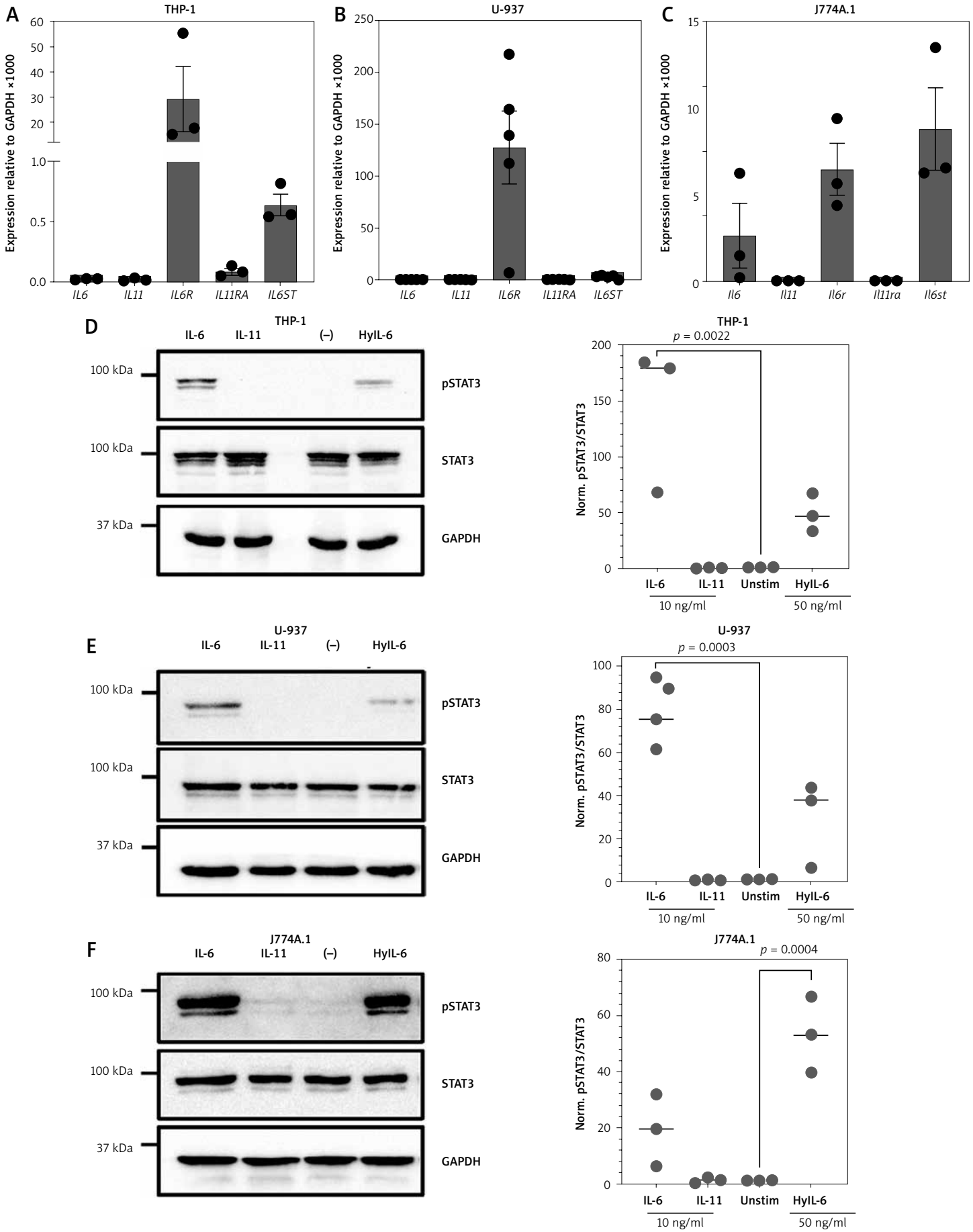

Fig. 3. Monocyte-derived immune cell lines express high levels of *IL6R* and respond to IL-6 classic and trans-signaling. **A-B**) Expression of *IL6*, *IL11*, *IL6R*, *IL11RA* and *IL6ST* was analyzed via quantitative real time polymerase chain reaction (qRT-PCR) in THP-1 cells (**A**) and U-937 cells (**B**). Expression of *Il6*, *Il11*, *Il6r*, *Il11ra* and *Il6st* was analyzed via qRT-PCR in J774.A1 cells. Data are shown as mean ± SD relative to GAPDH expression and are derived from three independent experiments (**C**). Serum-starved (**D**) THP-1 cells, (**E**) U-937 cells and (**F**) J774.A1 cells were either stimulated with IL-6, IL-11, or Hyper-IL-6 for 15 min or left unstimulated. Afterwards, cells were lysed and phosphorylation of the transcription factor STAT3 was analyzed by western blot. STAT3 and GAPDH were analyzed to verify equal loading. One representative experiment is shown, and the corresponding densitometric analysis of the pSTAT3/STAT3 ratio (mean ± SD, $n = 3$) is shown next to the western blots. Statistical analysis was performed using one-way ANOVA following Dunnett's multiple comparison test. Here, all stimulated conditions were compared to the unstimulated cells and in case of statistical significance, the *p*-value is shown above the respective bracket. All other comparisons were not significantly different

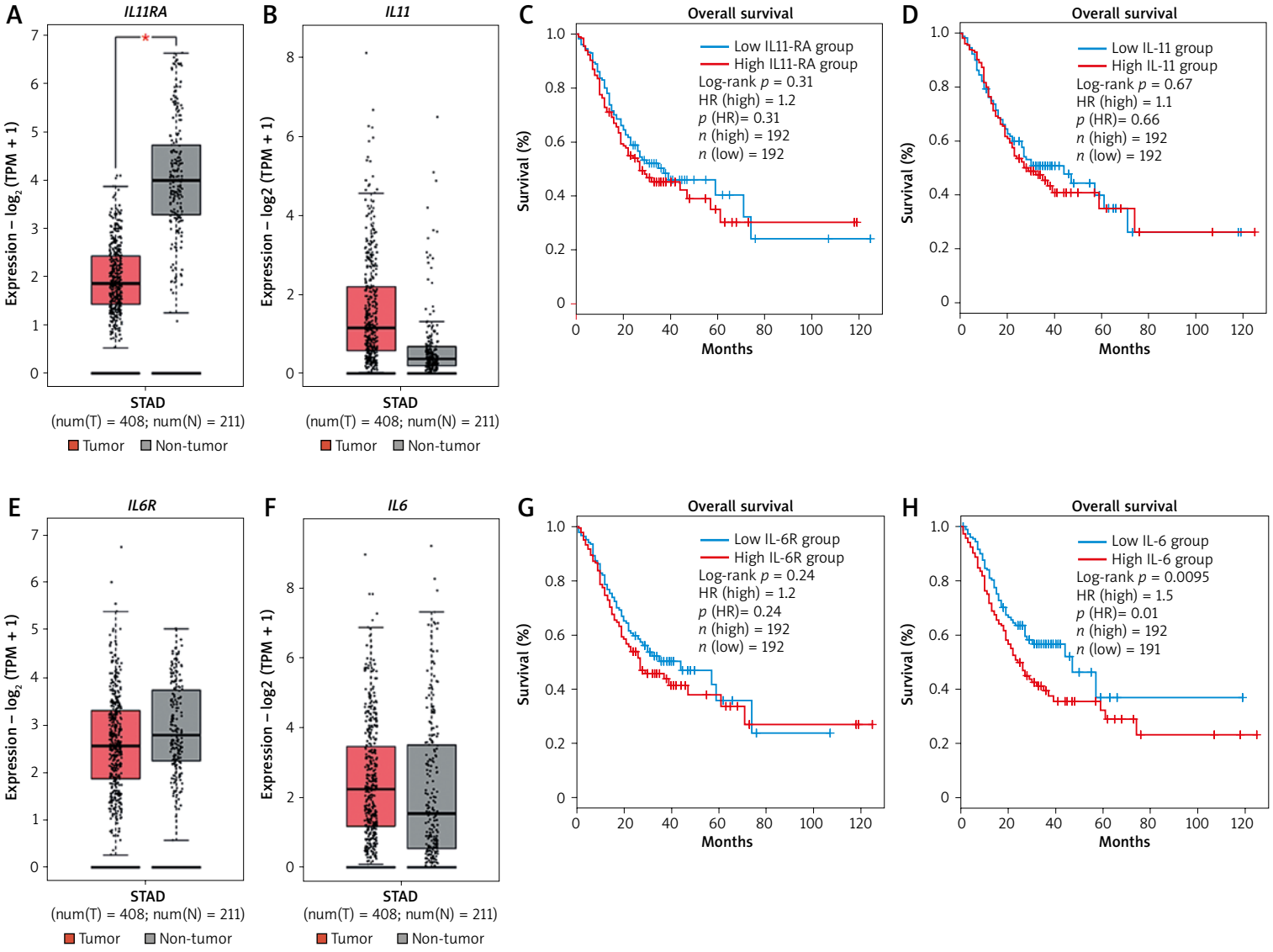

Fig. 4. Expression of *IL6* in gastric tumors is associated with patient overall survival. **A, B**) Expression levels of *IL11RA* (**A**) and *IL11* (**B**) in gastric tumor samples (red) and non-tumor control samples (gray). **C, D**) Tumor samples were divided into two groups (high/low) depending on their expression of *IL11RA* (**C**) or *IL11* (**D**) and overall survival was analyzed for both groups. Expression levels of *IL6R* (**E**) and *IL6* (**F**) in gastric tumor samples (red) and non-tumor control samples (gray). Tumor samples were divided into two groups (high/low) depending on their expression of *IL6R* (**G**) or *IL6* (**H**) and overall survival was analyzed for both groups

ing in recent years, this is primarily due to a reduction in *Helicobacter pylori* infections, one of the major causes of gastric tumorigenesis. Curative treatment, especially of patients with further progressed disease, is still difficult with the currently available options. Thus, the identification of novel therapeutic targets that can be used to either prevent or treat gastric tumors is of high relevance.

In the present study, we found that three gastric cancer cell lines express high amounts of gp130, the shared signal-transducing receptor of the IL-6 family of cytokines, rather low levels of the IL-6R and basically no IL-11R. Accordingly, these cell lines respond to stimulation with recombinant IL-6, but much better when stimulated with Hyper-IL-6, a fusion protein that mimics IL-6 trans-signaling. As it has been demonstrated before that STAT3, the transcription factor downstream of gp130, is crucial for gastric tumorigenesis [49, 50], these results suggest that IL-6, and especially IL-6 trans-signaling, are required for STAT3 activation in the investigated gastric cancer cell lines.

Rather surprisingly, we found no strong expression of IL-11R mRNA in the three tumor cell lines, and accordingly these cells did not respond to stimulation with IL-11, as seen by no phosphorylation of the transcription factor STAT3. This is at odds with previous studies that demonstrated a crucial, non-redundant role for IL-11 in gastric tumorigenesis [40, 41, 51]. This might be explained by the fact that most of the previous studies have been performed in mice and not in human samples. However, we also observed no strong effect of IL-11 on the murine cell line CLS-103. Otherwise, these differences could also be attributed to the fact that the cell lines that we used do not accurately depict the whole spectrum of gastric tumor cells that one would see, e.g. in a tumor specimen directly derived from cancer patients, and suggest that IL-11 signaling could be

more important in other cell types within the tumor or its environment, but not in the cancer cells themselves. Furthermore, *Helicobacter pylori* infection is not reflected in our cell culture model, an important factor that appears to contribute to IL-11-dependent gastric tumorigenesis [52].

Gastric tumors do not consist entirely of tumor cells, but also contain immune cells [8, 48]. Whether cytokines are derived from these invading immune cells, whether cytokines act on the immune cells or both is not entirely clear. We therefore analyzed whether different monocyte-derived immune cell lines respond to IL-6 and IL-11. Intriguingly, we observed high amounts of IL-6R and thus an efficient response to stimulation with exogenous IL-6, which is in line with previous reports that monocytes are among the cell types in the human body that can be activated by IL-6 *via* classic signaling [53–55]. Furthermore, these cells can be the source of sIL-6R to activate trans-signaling, as cells expressing IL-6R are likely candidates to release sIL-6R *via* proteolysis [22, 27]. Indeed, deletion of IL-6R *via* LysM-Cre resulted in 60% reduction of sIL-6R in mice, underscoring that these cells are important contributors to steady-state sIL-6R levels [56]. Again, we found no evidence for a prominent role of IL-11/IL-11R signaling in these cells.

Lastly, we analyzed differential gene expression data in gastric tumor patients compared to non-tumor controls and determined whether high or low expression levels would be predictive of overall survival. While we found no difference for *IL11* or *IL11RA*, low levels of *IL6* were associated with better survival. This corresponds to our *in vitro* data showing that gastric tumor cells respond better to IL-6 signaling compared to IL-11 signaling.

Conclusions

In summary, our data suggest that IL-6 contributes to gastric tumorigenesis. Our results highlight that the tumor cell lines we used do not accurately reflect the *in vivo* situation, as they are not responsive to IL-11. Future experiments should use primary tumor cells or organoids derived from primary tumors to better determine the individual contribution of IL-6 and IL-11 *via* classic and trans-signaling pathways in cancer and immune cells in gastric tumors.

Disclosures

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References

1. Sung H, Ferlay J, Siegel R L, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries, CA Cancer. J Clin 2021; 71: 209-249.
2. Robert-Koch-Institut (2024) Magenkrebs (Magenkarzinom). Available from: https://www.krebsdaten.de/Krebs/DE/Content/Krebsarten/Magenkrebs/magenkrebs_node.html.
3. Lindblad A, Kaucher S, Jaehn P, et al. The incidence of intestinal gastric cancer among resettlers in Germany – do resettlers remain at an elevated risk in comparison to the general population? Int J Environ Res Public Health 2020; 17: 9215.
4. Sitarz R, Skierucha M, Mielko J, Offerhaus GJA, Maciejewski R, Polkowski WP. Gastric cancer: epidemiology, prevention, classification, and treatment. Cancer Manag Res 2018; 10: 239-248.
5. Thrift AP, Wenker TN, El-Serag HB. Global burden of gastric cancer: epidemiological trends, risk factors, screening and prevention. Nat Rev Clin Oncol 2023; 20: 338-349.
6. Wu SL, Zhang Y, Fu Y, Li J, Wang JS. Gastric cancer incidence, mortality and burden in adolescents and young adults: a time-trend analysis and comparison among China, South Korea, Japan and the USA. BMJ Open 2022; 12: e061038.
7. Smyth EC, Nilsson M, Grabsch HI, van Grieken NC, Lordick F. Gastric cancer. Lancet 2020; 396: 635-648.
8. Lin Y, Pan X, Zhao L, et al. Immune cell infiltration signatures identified molecular subtypes and underlying mechanisms in gastric cancer. NPJ Genom Med 2021; 6: 83.
9. Garbers C, Hermanns H, Schaper F, et al. Plasticity and cross-talk of Interleukin 6-type cytokines. Cytokine Growth Factor Rev 2012; 23: 85-97.
10. Garbers C, Scheller J. Interleukin-6 and interleukin-11: same same but different. Biol Chem 2013; 394: 1145-1161.
11. Tanaka T, Narazaki M, Kishimoto T. IL-6 in inflammation, immunity, and disease. Cold Spring Harbor Perspect Biol 2014; 6: a016295.
12. Coulie PG, Cayphas S, Vink A, Uyttenhove C, Van Snick J. Interleukin-HP1-related hybridoma and plasmacytoma growth factors induced by lipopolysaccharide in vivo. Eur J Immunol 1987; 17: 1217-1220.
13. Elias J, Zheng T, Einarsson O, et al. Epithelial interleukin-11. Regulation by cytokines, respiratory syncytial virus, and retinoic acid. J Biol Chem 1994; 269: 22261-22268.
14. Heinrich PC, Behrmann I, Müller-Newen G, Schaper F, Graeve L. Interleukin-6-type cytokine signalling through the gp130/Jak/STAT pathway. Biochem J 1998; 334: 297-314.
15. Rodig SJ, Meraz MA, White MJ, et al. Disruption of the Jak1 gene demonstrates obligatory and nonredundant roles of the jaks in cytokine-induced biologic responses. Cell 1998; 93: 373-383.
16. Müllberg J, Schooltink H, Stoyan T, et al. The soluble interleukin-6 receptor is generated by shedding. Eur J Immunol 1993; 23: 473-480.
17. Garbers C, Jänner N, Chalaris A, et al. Species specificity of ADAM10 and ADAM17 proteins in interleukin-6 (IL-6) trans-signaling and novel role of ADAM10 in inducible IL-6 receptor shedding. J Biol Chem 2011;. 286: 14804-14811.
18. Riethmueller S, Somasundaram P, Ehlers JC, et al. Proteolytic origin of the soluble human IL-6R in vivo and a decisive role of n-glycosylation. PLoS Biol 2017; 15: e2000080.
19. Lokau J, Nitz R, Agthe M, et al. Proteolytic cleavage governs interleukin-11 trans-signaling. Cell Rep 2016; 14: 1761-1773.
20. Koch L, Kespohl B, Agthe M, et al. Interleukin-11 (IL-11) receptor cleavage by the rhomboid protease RHBDL2 induces IL-11 trans-signaling. FASEB J 2021; 35: e21380.
21. Lokau J, Garbers C. Biological functions and therapeutic opportunities of soluble cytokine receptors. Cytokine Growth Factor Rev 2020; 55: 94-108.
22. Schumertl T, Lokau J, Rose-John S, Garbers C. Function and proteolytic generation of the soluble interleukin-6 receptor in health and disease. Biochim Biophys Acta Mol Cell Res 2022; 1869: 119143.
23. Lokau J, Kespohl B, Kirschke S, Garbers C. The role of proteolysis in interleukin-11 signaling. Biochem Biophys Acta Mol Cell Res 2022; 1869: 119135.
24. Waage A, Brandtzaeg P, Halstensen A, Kierulf P, Espevik T. The complex pattern of cytokines in serum from patients with meningococcal septic shock. Association between interleukin 6, interleukin 1, and fatal outcome. J Exp Med 1989; 169: 333-338.
25. Garbers C, Heink S, Korn T, Rose-John S. Interleukin-6: designing specific therapeutics for a complex cytokine. Nat Rev Drug Discov 2018; 17: 395-412.
26. Garbers C, Aparicio-Siegmund S, Rose-John S. The IL-6/gp130/STAT3 signaling axis: recent advances towards specific inhibition. Curr Opin Immunol 2015; 34: 75-82.
27. Rose-John S, Jenkins BJ, Garbers C, Moll JM, Scheller J. Targeting IL-6 trans-signalling: past, present and future prospects. Nat Rev Immunol 2023; 23: 666-681.
28. Zhang S, Chen B, Wang B, et al. Effect of induction therapy with olamkicept vs placebo on clinical response in patients with ac-

tive ulcerative colitis: a randomized clinical trial. JAMA 2023; 329: 725-734.
29. Schreiber S, Aden K, Bernardes JP, et al. Therapeutic IL-6 trans-signalling inhibition by olamkicept (sgp130Fc) in patients with active inflammatory bowel disease. Gastroenterology 2021; 160: 2354-2366.e11.
30. Schulte DM, Waetzig GH, Schuett H, et al. Case report: arterial wall inflammation in atherosclerotic cardiovascular disease is reduced by olamkicept (sgp130Fc). Front Pharmacol 2022; 13: 758233.
31. Steadman T, O'Reilly S. Elevated interleukin-11 in systemic sclerosis and role in disease pathogenesis. J Dermatol 2023; 50: 1255-1261.
32. Schafer S, Viswanathan S, Widjaja AA, et al. IL11 is a crucial determinant of cardiovascular fibrosis. Nature 2017; 552: 110-115.
33. Karin M, Greten FR. NF-kappaB: linking inflammation and immunity to cancer development and progression. Nat Rev Immunol 2005; 5: 749-759.
34. Giraldez MD, Carneros D, Garbers C, Rose-John S, Bustos M. New insights into IL-6 family cytokines in metabolism, hepatology and gastroenterology. Nat Rev Gastroenterol Hepatol 2021; 18: 787-803.
35. Jackson C, Judd L, Menheniott T, et al. Augmented gp130-mediated cytokine signalling accompanies human gastric cancer progression. J Pathol 2007; 213: 140-151.
36. Ellmark P, Ingvarsson J, Carlsson A, Lundin BS, Wingren C, Borrebaeck CA. Identification of protein expression signatures associated with Helicobacter pylori infection and gastric adenocarcinoma using recombinant antibody microarrays Mol Cell Proteomics 2006; 5: 1638-1646.
37. Judd LM, Alderman BM, Howlett M, et al. Gastric cancer development in mice lacking the SHP2 binding site on the IL-6 family co-receptor gp130. Gastroenterology 2004; 126: 196-207.
38. Tebbutt N, Giraud A, Inglese M, et al. Reciprocal regulation of gastrointestinal homeostasis by SHP2 and STAT-mediated trefoil gene activation in gp130 mutant mice. Nat Med 2002; 8: 1089-1097.
39. Jenkins BJ, Grail D, Nheu T, et al. Hyperactivation of Stat3 in gp130 mutant mice promotes gastric hyperproliferation and desensitizes TGF-beta signaling. Nat Med 2005; 11: 845-852.
40. Ernst M, Najdovska M, Grail D, et al. STAT3 and STAT1 mediate IL-11-dependent and inflammation-associated gastric tumorigenesis in gp130 receptor mutant mice. J Clin Invest 2008; 118: 1727-1738.
41. Balic JJ, Garbers C, Rose-John S, Yu L, Jenkins BJ. Interleukin-11-driven gastric tumourigenesis is independent of trans-signalling. Cytokine 2017; 92: 118-123.
42. Tang Z, Kang B, Li C, Chen T, Zhang Z. GEPIA2: an enhanced web server for large-scale expression profiling and interactive analysis. Nucleic Acids Res 2019; 47: W556-W560.
43. Fischer M, Goldschmitt J, Peschel C, et al. A bioactive designer cytokine for human hematopoietic progenitor cell expansion. Nat Biotechnol 1997; 15: 142-145.
44. Mackiewicz A, Schooltink H, Heinrich PC, Rose-John S. Complex of soluble human IL-6-receptor/IL-6 up-regulates expression of acute-phase proteins. J Immunol 1992; 149: 2021-2027.
45. Lokau J, Göttert S, Arnold P, et al. The SNP rs4252548 (R112H) which is associated with reduced human height compromises the stability of IL-11. Biochim Biophys Acta 2017; 1865: 496-506.
46. Agthe M, Garbers Y, Grötzinger J, Garbers C. Two N-linked glycans differentially control maturation, trafficking and proteolysis, but not activity of the IL-11 receptor. Cell Physiol Biochem 2018; 45: 2071-2085.
47. Kespohl B, Hartig R, Garbers Y, Lokau J, Garbers C. Coding variants of the interleukin-11 receptor with reduced protein maturation show protease-dependent trans-signaling and transduce normal STAT3 signaling. Genes Dis Press 2022; 10: 373-376.
48. Sukri A, Hanafiah A, Kosai NR. The roles of immune cells in gastric cancer: anti-cancer or pro-cancer? Cancers (Basel) 2022; 14: 3922.
49. Balic JJ, Saad MI, Dawson R, et al. STAT3-mediated upregulation of the AIM2 DNA sensor links innate immunity with cell migration to promote epithelial tumourigenesis. Gut 2022; 71: 1515-1531.
50. Dawson RE, Deswaerte V, West AC, et al. STAT3-mediated upregulation of the AIM2 DNA sensor links innate immunity with cell migration to promote epithelial tumourigenesis. Gut 2022; 71: 1515-1531.
51. Buzzelli JN, O'Connor L, Scurr M, et al. Overexpression of IL-11 promotes premalignant gastric epithelial hyperplasia in isolation from germline gp130-JAK-STAT driver mutations. Am J Physiol Gastrointest Liver Physiol 2019; 316: G251-G262.
52. Sabry D, Abdelaleem OO, Hefzy EM, et al. Interplay between helicobacter pylori infection, interleukin-11, and leukemia inhibitory factor in gastric cancer among Egyptian patients. J Interferon Cytokine Res 2018; 38: 517-525.
53. Chalaris A, Garbers C, Rabe B, Rose-John S, Scheller J. The soluble Interleukin 6 receptor: generation and role in inflammation and cancer. Eur J Cell Biol 2011; 90: 484-494.
54. Scheller J, Chalaris A, Schmidt-Arras D, Rose-John S. The pro- and anti-inflammatory properties of the cytokine interleukin-6. Biochim Biophys Acta 2011; 1813: 878-888.
55. Wolf J, Rose-John S, Garbers C. Interleukin-6 and its receptors: a highly regulated and dynamic system. Cytokine 2014; 70: 11-20.
56. McFarland-Mancini M, Funk H, Paluch A, et al. Differences in wound healing in mice with deficiency of IL-6 versus IL-6 receptor. J Immunol 2010; 184: 7219-7228.

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