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Short communication

Interleukin-11-driven gastric tumourigenesis is independent of trans-signalling

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ABSTRACT

Deregulated gp130-dependent STAT3 signalling by the pleiotropic cytokine interleukin (IL)-11 has been implicated in the pathogenesis of gastric cancer (GC), the third most common cancer worldwide. While the IL-11-gp130-STAT3 signalling axis has traditionally been thought to exclusively use the membrane-bound IL-11 receptor (mIL-11R), recent evidence suggests that mIL-11R can be proteolytically cleaved to generate a soluble form (sIL-11R) which can elicit trans-signalling. Since the role of IL-11 trans-signalling in disease pathogenesis is unknown, here we have employed the IL-11-driven *gp130*$^{F/F}$ spontaneous model of GC to determine whether IL-11 trans-signalling promotes gastric tumourigenesis. sIL-11R protein was detectable in gastric tissue from GC patients, and sIL-11R levels were elevated in tumours of *gp130*$^{F/F}$ mice compared to matched non-tumours. Among candidate proteases associated with the generation of sIL-11R, ADAM10 and the related metalloprotease ADAM17 were significantly upregulated in tumours of both *gp130*$^{F/F}$ mice and GC patients compared to matched non-tumour tissues. The genetic blockade of IL-11 trans-signalling in *gp130*$^{F/F}$ mice upon the transgenic over-expression of the trans-signalling antagonist, sgp130Fc, failed to suppress gastric inflammation and associated tumour growth, and also had no effect on reducing hyper-activated STAT3 levels. Furthermore, a non-essential role for ADAM17 in IL-11-driven gastric tumourigenesis was supported by the observation that the tumour burden was unaffected in *gp130*$^{F/F}$:*Adam17*$^{ex/ex}$ mice in which ADAM17 expression levels have been substantially reduced. Collectively, these findings suggest that classic signalling rather than trans-signalling is the mode by which IL-11 promotes gastric tumourigenesis.

1. Introduction

Gastric cancer (GC) is the third most lethal cancer worldwide, and there is a strong link with deregulation of the immune system following infection by pathogenic microbes (i.e. *Helicobacter pylori*) leading to a cancerous cascade marked by chronic gastric inflammation (gastritis), atrophy, intestinal metaplasia, dysplasia and ultimately neoplasia [1]. The immunomodulatory cytokine interleukin (IL)-11, a member of the IL-6 cytokine family, is historically known for its anti-inflammatory actions, and yet has recently gained attention for its role in promoting inflammation-associated cancers of the gastrointestinal tract, in particular GC. For instance, elevated IL-11 expression levels are a common feature in tumours of GC patients, and are associated with the progression of tumours [2–4]. Furthermore, we have previously reported a causal role for IL-11 in promoting gastric tumourigenesis, whereby disruption of IL-11 signalling upon genetic ablation of the IL-11 receptor alpha subunit (IL-11R) in the *gp130*$^{F/F}$ spontaneous mouse model for GC prevents the onset of gastric tumours [5].

IL-11, along with the related cytokine IL-6, executes its biological actions via the ubiquitously-expressed common

Abbreviations: ADAM, a disintegrin and metalloprotease; EGFR, epidermal growth factor receptor; ELANE, elastase, neutrophil expressed; ERK, extracellular signal-regulated kinase; GC, gastric cancer; gp130, glycoprotein 130; IL-11, interleukin-11; JAK, janus kinase; MAPK, mitogen-activated protein kinase; mTOR, mechanistic target of rapamycin; SHP, src homology region 2 domain-containing phosphatase; PI3K, phosphoinositide 3-kinase; PRTN3, proteinase 3; STAT, signal transducer and activator of transcription; TCGA, The Cancer Genome Atlas; TNFα, tumour necrosis factor alpha.

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signal-transducing receptor beta subunit gp130, which forms a homodimer upon IL-11 or IL-6 binding to their respective alpha receptor subunits [6]. The dimerization of gp130 leads to activation of receptor-associated JAK tyrosine kinases, which tyrosine phosphorylate gp130 and promote the recruitment and subsequent activation of the latent transcription factors STAT3 and STAT1, mTORC1 and the SHP2 phosphatase, the latter of which facilitates activation of the ERK MAPK and PI3K/Akt pathways [6]. IL-6 signals via 2 distinct modes: classic signalling via its membrane-bound (m) IL-6 receptor (mIL-6R), and trans-signalling via a naturally-occurring soluble (s) IL-6R [7,8]. The recent development of inhibitors to specifically target either classic signalling or trans-signalling has implicated trans-signalling as the pathological mode by which IL-6 drives numerous inflammatory diseases and cancer, which is largely due to the expansion of cell types within the body that can respond to IL-6 signals [7,8]. Notably, a naturally-occurring soluble form of IL-11R has recently been discovered, although the pathophysiological consequences of sIL-11R-driven IL-11 trans-signalling remain unknown [9].

The 2 metalloproteases ADAM10 and ADAM17 are the major sheddases for the proteolytic release of sIL-6R [10], whereas IL-11R is the primary proteolytic substrate of ADAM10, as well as the proteases neutrophil elastase and proteinase 3 [9]. In human GC, increased ADAM10 and ADAM17 expression is associated with disease progression and poor prognosis [11,12], and recently ADAM10 was reported to promote gastric tumourigenesis in mice [13]. However, due to the wide range of additional substrates, which are collectively processed by ADAM10 and ADAM17, including EGFR ligands, Notch and TNFα, which are also implicated in inflammation and carcinogenesis, a definitive link between these proteases and IL-11 trans-signalling in the pathogenesis of GC remains unknown.

We have recently reported that the therapeutic compound sgp130Fc, which is a potent inhibitor of IL-6 trans-signalling [7,8], can also block IL-11 trans-signalling [9]. Here, we reveal that the transgenic over-expression of sgp130Fc in *gp130*$^{F/F}$ mice did not alleviate IL-11-driven gastric tumourigenesis. Furthermore, the genetic reduction of ADAM17 in *gp130*$^{F/F}$ mice failed to suppress the gastric tumour burden. Collectively, these findings support a non-essential role for both ADAM17 and trans-signalling in IL-11-driven gastric tumourigenesis.

2. Materials and methods

2.1. Mice

The *gp130*$^{F/F}$ and *gp130*$^{F/F}$:*sgp130Fc*$^{Tg/Tg}$ mice have been generated previously [5,14], and *gp130*$^{F/F}$:*Adam17*$^{ex/ex}$ mice were generated using *Adam17*$^{ex/ex}$ mice [15]. All mice were housed under specific pathogen-free conditions. Experiments were approved by the Monash University Animal Ethics Monash Medical Centre "A" Committee.

2.2. Human tissue collection

Gastric biopsies were collected from GC patients enrolled at Xin Hua Hospital (n = 15; Shanghai, China) undergoing surgical resection for clinical indications (Supplementary Table 1). Biopsies were either snap-frozen in liquid nitrogen or stored in 10% formalin, the latter for histopathological assessment. Full and informed consent was obtained from all patients, and biopsy collections were approved by the Xin Hua Hospital Ethics Committee.

2.3. RNA isolation and gene expression analysis

Total RNA extraction and cDNA preparation from mouse and human gastric tissues for quantitative RT-PCR (qPCR) expression analyses of individual genes were performed as before [5,6]. Sequence information for primers against mouse and human genes are listed in Supplementary Table 2.

Gene expression data and clinical information from The Cancer Genome Atlas (TCGA) GC database were obtained from the open access TCGA data portal (https://tcga-data.nci.nih.gov/tcga/dataAccessMatrix.htm). The alignment of sample identifiers yielded 262 primary tumour cases. We used the reads per kilobase of exon model per million mapped reads (RPKM) to quantify expression levels of indicated genes from RNA sequencing (RNA-Seq) data generated from each GC patient within the TCGA. Gene expression data was also available for tumour and matched non-tumour tissues from 26 GC patients.

2.4. Protein extraction, ELISA and immunoblotting

Mouse sera, and total protein lysates from snap-frozen mouse and human gastric tissues were subjected to ELISA and immunoblotting. The sgp130 ELISA was purchased from R&D systems (Minneapolis, MN). Immunoblotting was performed with antibodies against total STAT3 and phosphotyrosine (pY)-STAT3 (Cell Signaling Technology, Denver, MA), and IL-11R (Santa Cruz Biotechnology, Santa Cruz, CA). Protein bands were visualized using the Odyssey Infrared Imaging System (LI-COR, Lincoln, NE) and quantified using the ImageJ program (nih.gov). Antibody against α-Tubulin was purchased from Sigma-Aldrich (St. Louis, MO).

2.5. Histopathology and immunohistochemistry

The preparation of sections from formalin-fixed, paraffin-embedded mouse stomachs for H&E-staining and subsequent histopathological assessment of inflammation and tumours was performed as before [5,6]. Immunohistochemical staining to identify immune cells (anti-CD45; BD Biosciences, San Jose, CA) was detected with the Liquid Diaminobenzidine (DAB) Substrate Chromogen System (DakoCytomation, Carpinteria, CA). Sections were counterstained with hematoxylin.

2.6. Statistical analyses

Statistical analyses were performed using GraphPad Prism for Windows version 6.0. Paired *t*-tests were used to analyse normally-distributed data among sample groups, and Mann-Whitney tests for abnormally-distributed data, where appropriate. Data are expressed as the mean ± standard error of the mean (SEM), and $P < 0.05$ was considered statistically significant.

3. Results

3.1. Differential expression of sIL-11R, ADAM10 and ADAM17 in tumours of human GC patients and *gp130*$^{F/F}$ mice

Among genes encoding sheddases implicated in the generation of sIL-11R, expression of *ADAM10* and *ADAM17*, but not *ELANE* (encoding neutrophil elastase) and *PRTN3* (encoding proteinase 3), was significantly increased in tumour (~2-fold) compared to non-tumour tissue from TCGA GC patients (n = 262; Fig. 1A and Supplementary Table 1). Furthermore, in paired tumour and matched non-tumour tissues from TCGA (n = 26) and Chinese GC cohorts (n = 15), *ADAM10* expression was elevated in tumours from 22/26 (84.6%) and 8/15 (53.3%) patients, while *ADAM17* was increased in 25/26 (96.1%) and 11/15 (73.3%) patients, respectively (Fig. 1B, C). Notably, sIL-11R protein was strongly expressed in