TACE-dependent amphiregulin release is induced by IL-1β and promotes cell invasion in fibroblast-like synoviocytes in rheumatoid arthritis

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Abstract

Objectives. The aims of this study were to investigate the expression of amphiregulin (AREG) and TNF-α-converting enzyme (TACE) in fibroblast-like synoviocytes from humans with RA (FLS-RA) when stimulated with proinflammatory cytokines and to explore whether AREG plays a role in RA.

Methods. The effects of cytokines on the expression of AREG and TACE in FLS-RA were measured by quantitative RT-PCR and western blotting. Blockade of IL-1β-mediated pathways was used to verify the involvement of intracellular signal pathways in the induction of AREG and TACE. TAPI-1 and TACE short hairpin RNA (shRNA) infection were used to identify the role of TACE in IL-1β-induced AREG secretion and shedding. AREG-induced production of MMP-1 and cadherin-11 in FLS-RA were measured by ELISA or western blotting. The effect of AREG on FLS-RA invasion was examined using a Transwell invasion assay.

Results. IL-1β, but not other tested cytokines, increased the expressions of AREG mRNA and protein in a dose-responsive and time-dependent manner in FLS-RA. IL-1β induced AREG expression via p38 MAPK, NF-κB, JNK and ERK1/2 signalling pathways and induced TACE expression via PI3K, p38MAPK and NF-κB signalling pathways in FLS-RA. TACE mediated AREG secretion and shedding. EGFR (ErbB1) and Her-2 (ErbB2) were expressed in FLS-RA, and AREG increased MMP-1 and cadherin-11 expression in FLS-RA. AREG promoted the FLS-RA invasion ability.

Conclusion. AREG and TACE expression were up-regulated by IL-1β and their activations on FLS-RA lead to the matrix degradation by inducing MMP-1 and cadherin-11 production. TACE activity is necessary for IL-1β-induced AREG release. Our results demonstrate that IL-1β-induced AREG release may be involved in the pathogenesis of RA.

Key words: rheumatoid arthritis, fibroblast-like synoviocyte, IL-1β, amphiregulin.

Introduction

RA is a chronic immune disorder that affects the joints and promotes joint destruction [1]. Synovial hyperplasia is a hallmark of RA, and synovial fibroblasts play an important role in providing an inflammatory microenvironment by producing proinflammatory factors or recruiting immune cells [2]. Growth factors, including PDGF, epidermal growth factor (EGF) [3] and acidic and basic fibroblast growth factors (FGFs) [4], and cytokines, including IL-1, TGF-β [5], TNF-α [6] and IL-6 [7] are the molecules attributed to proliferation of synovial fibroblasts. Amphiregulin (AREG) is a member of the EGF family [8]. AREG is a 252-amino acid residue transmembrane precursor protein. To become active, AREG must be proteolytically cleaved to the mature form and released [9]. Many reports indicate that TNF-α-converting enzyme (TACE) is a major cleavage enzyme involved in the release of mature AREG [10, 11]. The EGF receptor (EGFR) is the receptor for AREG binding and AREG bioactivity requires activation of the EGFR pathway [12]. Physiologically AREG plays a role in the proliferation of normal cells, including fibroblasts, urothelial cells, normal human keratinocytes and...
human lung bronchial epithelial cells [13, 14]. AREG is also involved in physiological processes such as mammary gland development, bone formation, nerve regeneration and blastocyst implantation [15, 16]. However, because AREG may promote the proliferation and invasiveness of cancer cells, its overexpression is thought to be pathological and has been found in a variety of cancers, including breast, colon, lung, prostate, pancreatic, ovarian, skin and squamous cell carcinoma [17, 18]. AREG is also overexpressed in some autoimmune diseases such as SLE, psoriasis, idiopathic thrombocytopenic purpura and RA, and its overexpression may increase the production of proinflammatory cytokines, IL-6 and IL-8, which may increase the severity of disease [19, 20].

Although one report showed that AREG is highly expressed in RA patients [20], the relative contribution of proinflammatory cytokines to AREG expression in fibroblast-like synoviocytes in humans with RA (FLS-RA) and the downstream effects of AREG on the invasiveness of FLS-RA have not been examined. Furthermore, the relative contributions of proinflammatory cytokines to AREG expression in FLS-RA and the downstream effects of blockade of intracellular signalling on AREG production also have not been studied.

Here we evaluated AREG shedding and release in response to TACE activation. We investigated the role of AREG in the production of MMP-1 and cadherin-11 and the invasiveness of FLS-RA. We found that AREG is up-regulated by IL-1β in FLS-RA, TACE is a candidate enzyme for IL-1β-induced AREG secretion and AREG may play an important role in increasing the invasion ability of FLS-RA.

Materials and methods

Patients’ samples

Synovial tissues from three RA and three OA patients were obtained after joint replacement surgery. The tissues were fixed in 4% paraformaldehyde overnight and paraffinized. All patients met the ACR (formerly the American Rheumatism Association) 1987 revised criteria for RA and OA [21]. Informed consent was obtained from each patient and the study was approved by the ethics committee of the Tri-Service General Hospital, National Defense Medical Center, Taiwan, Republic of China.

Immunohistochemistry analysis

Paraffinized human knee-joint synovial tissues sections, 3-μm thick, were deparaffinized, placed in 0.01 M sodium citrate buffer (pH 6.0) and heated twice for 5 min in a microwave oven. After inactivation of endogenous peroxidase with 0.5% metaperiodic acid in PBS for 10 min, sections were incubated with 10% horse serum in PBS for 1 h. Sections were incubated at 4°C overnight with 100× diluted primary goat anti-human AREG antibody (R&D Systems, Minneapolis, MN, USA). Sections were incubated with the Dako Envision Plus system to amplify the signal after washing with PBS. The signals were finally developed with diaminobenzidine (Nichirei, Tokyo, Japan).

FLS-RA culture

Synovial fibroblasts were isolated from knee biopsies of four RA patients at the time of total joint replacement surgery. Synoviums were minced and digested in 0.2% collagenase in DMEM containing 10% FBS (HyClone Laboratories, Logan, UT, USA) for 6 h at 37°C.

Primary FLS-RA were maintained in 10% FBS, 2 mM L-glutamine, 3 mM NaHCO3, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37°C (5% CO2) in a humidified air atmosphere. Cells were used for test from passages 3–8.

Cell treatment

To examine the effect of cytokines on AREG production, FLS-RA were in serum-free DMEM for 24 h, then cells exposed to various proinflammatory cytokines (R&D), such as TNF-α (10 ng/ml), IL-17 (100 ng/ml), IL-12 (100 ng/ml), IL-23 (100 ng/ml) and IL-1β (1 ng/ml), or Th1 cytokine, IFN-γ (100 ng/ml) in fresh DMEM and 0.2% LAH medium for 18 h. Detection of AREG mRNA expression used real-time RT-PCR.

Time course and dose response of IL-1β effect on AREG and TACE expression

To examine the effect of IL-1β on AREG and TACE production, 1 × 106 FLS-RA cells were placed in serum-free DMEM for 24 h, then cells exposed to IL-1β (1 ng/ml) in serum-free DMEM for 3 h, 6 h, 12 h, 24 h and 48 h or 18 h with various concentrations of IL-1β (0, 0.01, 1 and 10 ng/ml). Total RNA was isolated from FLS-RA and the expression of AREG and TACE was analysed by real-time RT-PCR. Western blotting was used to determine the time-dependent expression of AREG and TACE protein in FLS-RA stimulated with 1 ng/ml IL-1β for different time points and the dose-dependent expression of AREG protein stimulated with various concentrations of IL-1β.

The effect of signal pathway inhibitors in IL-1β induced AREG and TACE gene expression

To establish a cause-and-effect relationship between IL-1β-mediated signal pathways and AREG or TACE gene expression, we used inhibitors for NF-κB (PDTC; Sigma), p38MAPK (SB203580; Calbiochem), ERK1/2 (PD98059; Calbiochem) and PI3K (LY294002; Calbiochem). A total of 1 × 106 FLS-RA in serum-free DMEM were pre-treated with various concentrations of inhibitors for 1 h and then treated with IL-1β for another 18 h. AREG and TACE gene expression was determined by quantitative PCR (qPCR).

RNA interference

TACE small hairpin RNA containing lentiviral vectors was obtained from the National RNA Interference (RNAi) Core Facility and lentiviruses were generated according to the standard protocol of the National RNAi Core Facility. Recombinant lentivirus-infected cells were enriched by puromycin selection for 7–10 days.
The effect of TACE in IL-1β-induced AREG secretion

Cells were pre-treated with TACE inhibitor (TAPI-1; Calbiochem) for 1 h followed by stimulation with 1 ng/ml IL-1β for 24 h and the cell supernatants were collected for AREG secretion assay by ELISA. FLS-RA were transduced with lentiviruses at an multiplicity of infection (MOI) of ~2. After transduction, cells were selected by puromycin for 7 days. The knockdown efficiency was checked by Western blotting. The TACE knockdown cells were stimulated with 1 ng/ml IL-1β for 24 h and the cell supernatants were collected for AREG secretion assay by ELISA.

Total RNA extraction, semi-quantitative RT-PCR and qPCR

Total cellular RNA was extracted from cells with different treatment by using the NucleoSpin RNAII kit (Macherey-Nagel, Düren, Germany). For RT-PCR analysis, 1 μg of total RNA was converted into cDNA using oligo(dT) and reverse transcriptase (Applied Biosystems). The PCR primers and conditions are listed in the supplementary data available at Rheumatology Online.

qPCR was performed on an ABI-Prism 7000 PCR cycler (ABI) and as described using the following Taqman primer sets: AREG (Hs00950669_m1), TACE (Hs01041915_m1) and GAPDH (Hs99999905_m1).

Effect of AREG on MMP-1 and cadherin-11 expression in FLS-RA

A total of 3.5 × 10^5 FLS-RA were in serum-free DMEM. After 24 h the medium was replaced with serum-free medium containing various doses of AREG (0, 1, 10, 100 and 1000 ng/ml). After 48 h the supernatants for the MMP-1 assay and cell lysate for cadherin-11 were collected and analysed. MMP-1 was analysed by ELISA using R&D assay kits according to the manufacturer’s protocol. Cadherin-11 was analysed by western blotting.

Western blot analysis

Total proteins were isolated from the cell extracts (35 μg of total protein per lane) using 10% SDS-PAGE. The protein separated on the gels was transferred onto polyvinylidene fluoride (PVDF) membranes (Amersham). The blots were stained with anti-AREG (R&D), anti-TACE (Millipore) or anti-cadherin-11 (Invitrogen) antibody and followed by a secondary staining with peroxidase-conjugated anti-goat IgG (Sigma), anti-mouse IgG (Jackson) or anti-rabbit IgG (Jackson). The protein bands on the filter were visualized using an ECL system (Amersham).

Invasion assay

A Transwell system (Coming, Lowell, MA, USA) was coated with Matrigel (37.5 μg/well) on the top of the polycarbonate filter insert. FLS-RA (2 × 10^4 cells/well) were seeded in 200 μl serum-free DMEM and incubated without or with AREG (1, 10, 100 ng/ml) at 37°C in the upper chamber of a Transwell system. The lower chambers were filled with 900 μl DMEM with 10% FBS. After 48 h the assay was terminated by fixing the cells in 100% methanol. The Matrigel together with the cells on the upper side of the filter were scraped off. Invasion was determined by the cell numbers on the bottom side of filter.

Results

AREG expression in synovial tissue from RA patients

To evaluate AREG expression in human joints we performed immunohistochemical analyses using a specific antibody against AREG to localize AREG in the synovium and cartilage samples obtained from RA and OA patients. As shown in Fig. 1, AREG was expressed in the synovium of joints from RA patients, but not in OA synovium.

Effect of cytokines on AREG mRNA production by FLS-RA

Immunohistochemical analyses showed that synovial fibroblasts expressed AREG protein in RA samples (Fig. 1). Real-time PCR showed higher AREG mRNA levels in synovial tissues from RA patients compared with that from OA patients [20]. The mechanisms by which cytokines induce AREG expression in FLS-RA are still unknown. To examine the possible effects of cytokines on the expression of AREG mRNA, FLS-RA were treated with various proinflammatory cytokines, such as TNF-α, IL-17, IL-12, IL-23 and IL-1β or the Th1 cytokine, IFN-γ. As shown in Fig. 2, following stimulation with only IL-1β (1 ng/ml), the expression of AREG mRNA expression in FLS-RA cells was about 120-fold of the untreated control level. Other cytokines, including TNF-α (10 ng/ml), IL-17 (100 ng/ml), IL-12 (100 ng/ml), IL-23 (100 ng/ml) and IFN-γ (100 ng/ml), did not alter AREG mRNA production in FLS-RA.

Fig. 1 Immunohistological localization of AREG in synovium membrane with RA patients.
To determine which pathways mediate IL-1β-induced AREG expression, we used signalling inhibitors to examine the involvement of the signalling pathways. FLS-RA were incubated with signalling inhibitors for 1 h and then stimulated with 1 ng/ml IL-1β for another 18 h. Fig. 3E shows that inhibitors of the p38 MAPK (SB203580), JNK (SP600125) and ERK1/2 (PD98059) pathways significantly inhibited the IL-1β-induced expression of AREG mRNA in FLS-RA. AREG mRNA expression was decreased by 27%, 45%, 66% and 91%, respectively, compared with IL-1β stimulation alone. Pre-treatment with 25 µM PD98059 almost completely inhibited the IL-1β-induced AREG expression. In contrast, the PI3K inhibitor (LY294002) increased the IL-1β-induced expression of AREG by 50% compared with only IL-1β treated FLS-RA. These results indicate that the IL-1β-induced increase in AREG expression in FLS-RA involves the p38 MAPK, ERK1/2, JNK and NF-κB pathways, and that suggests the PI3K pathway might be an inhibitory mediator of AREG expression.

Dose-dependent effect of IL-1β on TACE expression

Because precursor AREG must be cleaved and shed by TACE to become a soluble and mature AREG, we investigated whether IL-1β-induced secretion of AREG is regulated by TACE. First, we determined the effect of IL-1β on TACE expression in FLS-RA. As shown in Fig. 4A, IL-1β increased the expression of TACE mRNA in a dose-dependent manner by 1- to 4-fold (P < 0.05). Western blotting analysis showed that the mature form of TACE protein was constitutively expressed in FLS-RA and that IL-1β induced TACE protein expression in a concentration-dependent manner (Fig. 4B).

Role of PI3K, p38 MAPK and NF-κB pathways in IL-1β-induced TACE expression

To examine the signalling pathways involved in IL-1β-induced TACE expression, we used inhibitors to identify which pathways are involved in IL-1β-induced TACE expression. FLS-RA were incubated with or without 1 ng/ml IL-1β in the presence or absence of inhibitors of PI3K (LY294002), p38 MAPK (SB203580), NF-κB (PDTC), JNK (SP600125) or ERK1/2 (PD98059). We measured changes in TACE mRNA expression using real-time RT-PCR. IL-1β-induced TACE transcription was significantly attenuated in FLS-RA by pre-treatment with the inhibitors of PI3K, p38 MAPK and NF-κB (P < 0.05), but not by the inhibitors of JNK and ERK1/2. These results suggest that activations of the PI3K, p38 MAPK and NF-κB pathways are involved in IL-1β-induced TACE expression (Fig. 4C).

Down-regulation of TACE expression reduces IL-1β-induced AREG expression in FLS-RA

Next we investigated the relationship between IL-1β-induced AREG secretion and TACE expression. We pre-treated FLS-RA with or without different concentrations (5, 10 or 20 µM) of TAPI-1, a specific TACE inhibitor, for 1 h and stimulated the cells with 1 ng/ml IL-1β for another 24 h. We used an ELISA to measure mature AREG expression in the cell supernatant. The results shown in Fig. 5A indicate that TAPI-1 blocked IL-1β-induced AREG secretion in FLS-RA in a dose-dependent manner. TAPI-1 exerted a maximum effect at a concentration of 20 µM and attenuated the IL-1β-induced secretion of AREG protein by 50% (P < 0.001). These data suggest that TACE is responsible for IL-1β-induced AREG shedding in FLS-RA (Fig. 5A).
To reconfirm the role of TACE in IL-1β-induced secretion of AREG, we used a specific human TACE short hairpin RNA (shRNA) to knock down TACE expression. Stable knockdown of TACE expression is mediated by a lentivirus-based shRNA vector (Fig. 5B). Knockdown of TACE expression in FLS-RA decreased the release of soluble AREG in response to IL-1β stimulation (Fig. 5C).

EGFR and HER-2 gene expression in FLS-RA
To evaluate the expression of EGF ligand-associated cellular receptors, we used primer sequences specific for the human EGFR, HER-2, HER-3 and HER-4 genes. RT-PCR showed constitutive expression of EGFR and HER-2 mRNA in primary FLS-RA from four RA patients (Fig. 6A). In contrast, HER-3 and HER-4 mRNA could not be detected in these cells.

AREG induced production of MMP-1 and cadherin-11 in FLS-RA and increased FLS-RA invasive ability
We next examined the pathological function of AREG in RA. Fig. 6B shows that AREG induced MMP-1 expression in a dose-dependent manner, with maximal effect at a
concentration of 100 ng/ml AREG (P < 0.001). Supplementary Fig. S1A, available at Rheumatology Online, demonstrates that AREG only weakly induced MMP-1 expression in fibroblast-like synoviocytes in humans with OA (FLS-OA). Western blot analysis showed that AREG increased cadherin-11 expression in FLS-RA but not in FLS-OA (Fig. 6C, and Supplementary Fig. S1B, available at Rheumatology Online). Using BD Bio-coat cell invasion chambers, we found that treatment of FLS-RA with 100 ng/ml AREG increased the percentage of invasive cells by 20%. These results suggest that AREG plays a pathological role in RA by increasing MMP-1 and cadherin-11 expression and the invasive ability of FLS-RA.

**Discussion**

RA is a chronic inflammatory disease whose hallmark is affliction of the joints, characterized by inflammation and proliferation of synovial tissue that causes invasion of synovial cells into articular cartilage [22]. Many cytokines participate in RA pathogenesis [23]. FLS in the synovial intimal lining are considered key effector cells in inflammation by producing inflammatory cytokines, growth factors, and MMPs [24, 25]. In RA joints, IL-1β and TNF-α are the most important proinflammatory cytokines that mediate joint destruction [26, 27]. Previous studies have indicated that EGF-related proteins, such as TGF-β and AREG, and EGF-related receptors, including EGFR and Her-2, are highly expressed in the RA synovium. These factors play important roles in promoting FLS-RA proliferation and in IL-6 and IL-8 secretion [20, 28]. There have been few reports on the factors regulating AREG production in cells. Inatomi et al. [29] reported that IL-1β and TNF-α strongly induced AREG mRNA expression in human colonic subepithelial myofibroblasts. TGF-β1 significantly induced AREG expression in lung fibroblasts [30]. Our results show that AREG protein is highly expressed in the RA synovium and that IL-1β, but not other proinflammatory cytokines, induces AREG mRNA and protein expression in a dose- and time-dependent manner in FLS-RA. Different cell types might have different regulation mechanisms for inducing AREG expression. The intracellular signalling pathways that induce AREG

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**Fig. 4** Effect of IL-1β on TACE expression in FLS-RA.

(A) Fibroblast-like synoviocytes from humans with RA (FLS-RA) were treated with IL-1β with concentrations from 0 to 10 ng/ml for 18 h for RNA analysis and for 24 h for protein analysis. RNA was extracted and expression of TNF-α-converting enzyme (TACE) was analysed by real-time RT-PCR. (B) TACE proteins were extracted then analysed using western blotting. (C) FLS-RA pre-treated with LY294002 (5 μM), SB203580 (15 μM), PDTC (50 μM), SP600125 (5 μM), PD98059 (25 μM) and U0126 (5 μM) for 1 h, then incubated with 1 ng/ml IL-1β. Analysis of TACE mRNA expression was done using real-time RT-PCR. Data for each sample were normalized to the GADPH mRNA level in each sample and normalized again between samples to the levels of AREG present in unstimulated inhibitor conditions. Data are means (s.e.m.) from three experiments with cells from three different RA patients. *P < 0.05 by Mann-Whitney U-test.
expression in FLS-RA have not been reported. Previous studies have reported that IL-1β activates MAPK and NF-κB pathways in other cell types [31, 32], therefore we used specific signal-transduction inhibitors to explore these pathways. Our results are the first to show that the p38 MAPK, NF-κB, JNK and ERK1/2 signalling pathways are essential for IL-1β-induced AREG expression in FLS-RA.

Some studies have reported that AREG is translated as a precursor transmembrane protein and must be cleaved by metalloproteases to yield the mature and secreted form. TACE is considered a candidate enzyme responsible for AREG maturation and secretion [33]. Several studies have reported that TACE expression is higher in RA joints compared with OA or normal joints [34]. Because of the pathophysiological importance of TACE-mediated shedding, several studies have examined whether abnormal TACE activity contributes to TNF-α action in RA pathogenesis [35, 36]; however, the mechanism involved in regulating TACE expression in FLS-RA remains unknown. Our results indicate that FLS-RA constitutively produces mature TACE and that IL-1β increases TACE expression in FLS-RA via PI3K, p38 MAPK and NF-κB signalling. Several studies have reported similar findings—for example, that hypoxia and TNF-α increase TACE expression in RA synovial cells. The increase in TACE expression in synovial cells might occur via the NF-κB pathway [37]. IL-1β up-regulates TACE expression in human neuroblastoma SK-N-SH cells [38]. VEGF increases the levels of TACE mRNA in murine retinal endothelial cells [39]. However, we are the first to identify the regulatory mechanisms underlying the IL-1β-mediated increase in TACE expression in FLS-RA.

The TACE-dependent release of AREG is a key step in the transactivation of EGFR in tobacco smoke-stimulated bronchial epithelial cells, in a TNF-α-stimulated human airway epithelial cell line and in cigarette smoke-exposed NCI-H292 cells [19, 33, 40]. To clarify the role of TACE in IL-1β-stimulated AREG secretion, we used a relatively selective inhibitor of TACE, TAPI-1, and specific knockdown of TACE with TACE shRNA before IL-1β stimulation in FLS-RA. We found that the IL-1β-induced AREG release in FLS-RA cells was inhibited by TAPI-1 and the specific TACE shRNA. These data suggest that IL-1β-mediated AREG secretion in FLS-RA cells is mediated by TACE activity. IL-1β-induced a dose-dependent increase in AREG expression followed by shedding and activation of the TACE protein,
and TACE appeared to be responsible for IL-1β-induced AREG shedding. Interestingly, activation of PI3K, p38 MAPK and NF-κB were involved in IL-1β-induced TACE expression, but PI3K was a negative regulatory pathway for IL-1β-induced AREG expression. The IL-1β-mediated effects on the p38 MAPK, NF-κB, JNK and ERK1/2 pathways were more strongly related to the increase in AREG mRNA expression compared with the PI3K pathway.

AREG contributes to cell proliferation in various cell types. AREG mRNA is expressed in a variety of human tumours, such as colorectal, breast and prostate cancers, thus AREG is considered a cancer biomarker. Various studies have indicated that AREG plays a major role in promoting cancer cell proliferation, motility and invasion [41, 42]. AREG also promotes FLS-RA proliferation (Supplementary Fig. S2, available at Rheumatology Online). Some studies have suggested that AREG overexpression may be associated with autoimmune diseases, including RA and SS, because AREG plays a critical role in the secretion of proinflammatory cytokines, which can induce hyperplasia and angiogenesis [19, 43]. One report suggested that AREG might affect IL-8 secretion in epithelial cells. Yamane et al. [20] demonstrated that AREG enhanced FLS-RA invasive activates. FLS-RA were seeded to the upper wells of Transwell chambers coated with 100 μg Matrigel and treated with different doses of AREG for 48 h. The average number of cells for the bottom of Transwells and data represent the mean [S.E.M.] of five independent experiments. The numbers of untreated cells were set 100%. *P < 0.05, **P < 0.001 by Mann-Whitney U-test.

Fig. 6 The effects of AREG on FLS-RA.

(A) Expression of the EGFR and HER-2 mRNA in Fibroblast-like synoviocytes from humans with RA (FLS-RA). FLS-RA obtained from four RA patients. RT-PCR was performed using specific primers for EGFR, HER-2 and GAPDH. (B) MMP-1 protein was analysed by an MMP-1 ELISA kit after stimulation without or with various concentrations of amphiregulin (AREG) for 48 h. (C) Cadherin-11 expression in FLS-RA was analysed by western blotting treated without and with (1, 10 and 100 ng/ml) AREG for 48 h. Cadherin-11 proteins were analysed by western blot using antibodies to cadherin-11 or β-actin. (D) AREG enhanced FLS-RA invasive activates. FLS-RA were seeded to the upper wells of Transwell chambers coated with 100 μg Matrigel and treated with different doses of AREG for 48 h. The average number of cells for the bottom of Transwells and data represent the mean [S.E.M.] of five independent experiments. The numbers of untreated cells were set 100%. *P < 0.05, **P < 0.001 by Mann-Whitney U-test.
recombinant human AREG dose-dependently induced MMP-1 and cadherin-11 expression in FLS-RA and increased the invasive potential of these cells by ~20% compared with controls. The mechanisms proposed to explain the effects of AREG on cellular invasion focus on altered expression of MMP-1 and cadherin-11. These results suggest a role for AREG in RA pathogenesis. This hypothesis is consistent with other reports that have demonstrated an effect of AREG on cancer cell invasion.

In conclusion, our results demonstrate that TACE and AREG expression in FLS-RA is up-regulated by IL-1β and that their activation might lead to matrix and cartilage degradation in RA. IL-1β-induced AREG may play an important role in the pathogenesis of RA.

Rheumatology key messages

- IL-1β-induced amphiregulin release in fibroblast-like synoviocyte-RA was dependent on TNF-α-converting enzyme activity.
- Understanding the role of amphiregulin in fibroblast-like synoviocyte-RA was a therapeutic target for RA.

Acknowledgements

We thank the National RNAi Core Facility (Academia Sinica, Taiwan) for providing shRNA constructs. F.-L.L. was supported by the National Science Council, Taiwan, Republic of China (NSC100-2811-B-016-012 and NSC101-2811-B-016-003). Study conception, design and writing: F.-L.L. and D.-M.C.; acquisition of data: F.-L.L. and C.-C.W.; analysis and interpretation of data: F.-L.L. and D.-M.C.

Funding: This work was supported by the National Science Council, Taiwan, Republic of China (99-2628-B-016-001-MY3 and 101-2314-B-016-024-MY3).

Disclosure statement: The authors have declared no conflicts of interest.

Supplementary data

Supplementary data are available at Rheumatology Online.

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