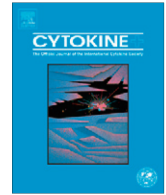
Cytokine 59 (2012) 218–222

Contents lists available at SciVerse ScienceDirect

Cytokine

journal homepage: www.elsevier.com/locate/issn/10434666



Short Communication

Variable modulation by cytokines and thiazolidinediones of the prototype Th1 chemokine CXCL10 in anaplastic thyroid cancer

Alessandro Antonelli [a,*], Silvia Martina Ferrari [a], Poupak Fallahi [a], Simona Piaggi [b], Andrea Di Domenicantonio [a], David Galleri [c], Libero Santarpia [d], Fulvio Basolo [c], Ele Ferrannini [a], Paolo Miccoli [c]

[a] Department of Internal Medicine, University of Pisa – School of Medicine, Via Roma 67, 56126 Pisa, Italy
[b] Department of Experimental Pathology, University of Pisa – School of Medicine, Via Roma 67, 56126 Pisa, Italy
[c] Department of Surgery, University of Pisa, Via Paradisa 2, 56126 Pisa, Italy
[d] Translational Research Unit, Department of Oncology, Hospital of Prato, Istituto Toscano Tumori, Piazza Ospedale 2, 59100 Prato, Italy

ARTICLE INFO

Article history:
Received 3 February 2012
Received in revised form 12 April 2012
Accepted 27 April 2012
Available online 25 May 2012

Keywords:
CXCL10
Thiazolidinediones
Anaplastic thyroid cancer
Cytokines
Chemokines

ABSTRACT

Until now, no data are present in literature about the prototype Th1 chemokine (C–X–C motif) ligand 10 (CXCL10) in anaplastic thyroid cancer (ATC).

This study aimed to test in "primary human ATC cells" (ANA) *vs* "normal thyroid follicular cells" (TFC): (a) CXCL10 secretion basally and after interferon (IFN)-γ and/or tumor necrosis factor (TNF)-α stimulation; (b) peroxisome proliferator-activated receptor (PPAR)-γ activation by thiazolidinediones, rosiglitazone or pioglitazone, on CXCL10 secretion, on proliferation and apoptosis in ANA.

We demonstrate that: (a) ANA, but not TFC, produced basally CXCL10, and did so in half of cases; (b) IFN-γ stimulated dose-dependently CXCL10, in ANA and TFC; (c) TNF-α did not induce CXCL10 secretion, in ANA and TFC; (d) IFN-γ + TNF-α induced a synergistic but variable release of CXCL10 in the different ANA preparations, while it was more reproducible in TFC; (e) rosiglitazone action on CXCL10 in ANA was inhibitory in 2/6, stimulatory in 1/6 and nil in 3/6, whereas it was inhibitory in TFC; (f) rosiglitazone inhibition of proliferation in ANA was not associated with the effect on CXCL10; (g) nuclear factor-κB and ERK1/2 were basally activated in ANA, increased by IFN-γ + TNF-α, and rosiglitazone inhibited that activation.

On the whole, the present data first show that ANA cells are able to produce CXCL10, basally and under the influence of cytokines. However, the pattern of modulation by IFN-γ, TNF-α or thiazolidinediones is extremely variable, suggesting that the intracellular pathways involved in the chemokine modulation in ATC have different types of deregulation.

© 2012 Elsevier Ltd. All rights reserved.

1. Introduction

A key role in cancer is played by members of the chemokine superfamily [1], however until now, little is known about chemokines in thyroid cancer.

It has been shown that both established cell lines and primary cultures of anaplastic thyroid cancer (ATC) overexpress CXC chemokine receptors 4 (CXCR4) [2]. In papillary thyroid cancer (PTC), upregulation of the chemokine (C–X–C motif) ligand (CXCL)10 has been shown [3–5]. More recently, it has been shown that a more than 10 times higher CXCL10 secretion has been induced by interferon (IFN)-γ + tumor necrosis factor (TNF)-α in PTC cells in comparison with normal thyroid follicular cells (TFC) [6].

However, until now, to our knowledge, no data are present in literature about the prototype T helper (Th1) chemokine CXCL10

Abbreviations: ANA, primary ATC cells cultures; ATC, anaplastic thyroid cancer; BCA, bicinchoninic acid; CXCL, chemokine (C–X–C motif) ligand; CXCR, CXC chemokine receptors; ELISA, enzyme-linked immunosorbent assay; EMA, European medicine agency; EMSA, electrophoretic mobility shift assay; FBS, fetal bovine serum; IE50, the concentrations of TZD required for 50% inhibition of growth; IFN, interferon; NIS, sodium/iodide symporter; PBS, phosphate buffered saline; PCR, polymerase chain reaction; PCR-SSCP, PCR single strand conformation polymorphism; POD, BM chemiluminiscene blotting substrate; PPAR, Peroxisome proliferator-activated receptor; PTC, papillary thyroid cancer; RGZ, rosiglitazone; TFC, thyroid follicular cells; Tg, thyroglobulin; Th, T helper; TNF, tumor necrosis factor; TPO, thyroperoxidase; TSH, thyroid stimulating hormone; TZDs, thiazolidinediones.

* Corresponding author at: Department of Internal Medicine, University of Pisa – School of Medicine, Via Roma 67, I-56100 Pisa, Italy. Tel.: +39 050 992318; fax: +39 050 553414.

E-mail addresses: alessandro.antonelli@med.unipi.it (A. Antonelli), sm.ferrari@int.med.unipi.it (S.M. Ferrari), poupak@int.med.unipi.it (P. Fallahi), simona.piaggi@dps.unipi.it (S. Piaggi), didomand@libero.it (A. Di Domenicantonio), galleridavid@hotmail.com (D. Galleri), lsantarpia@usl4.toscana.it (L. Santarpia), f.basolo@med.unipi.it (F. Basolo), ferranni@ifc.cnr.it (E. Ferrannini), p.miccoli@dc.med.unipi.it (P. Miccoli).

1043-4666/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.cyto.2012.04.042

in ATC, nor of peroxisome proliferator-activated receptor (PPAR)-γ activators effect on this chemokine.

The aims of this study were: (a) to evaluate CXCL10 in primary ATC cells cultures (ANA) with respect to the normal thyrocytes; (b) to test the effect of IFN-γ/TNF-α stimulation on the secretion of CXCL10 in ATC cells, in comparison with TFC; (c) to assess the effect of PPAR-γ activation on CXCL10 secretion and proliferation in these cell types.

2. Materials and methods

2.1. Patients source for thyroid tissue, ATC and TFC cell cultures

Surgical thyroid tissue was obtained from six patients with ATC at the time of surgery. In addition, normal thyroid tissue was obtained from six patients undergoing parathyroidectomy. The diagnosis was established on commonly accepted clinical, laboratory, and histological criteria [7–9]. Immunohistochemistry showed the absence of expression of thyroid stimulating hormone (TSH) receptor, thyroperoxidase (TPO), thyroglobulin (Tg) and Sodium/Iodide Symporter (NIS). Detection of *BRAF* mutations was performed using conventional methods previously described [6]. The study subjects gave their informed consent to the study, which was approved by the local ethical committee.

ATC cells [7–9] and TFC [10,11] cells were prepared as reported previously.

2.2. CXCL10 secretion assay

For CXCL10 secretion assays, 3000 cells were seeded onto 96-well plates in growth medium. After 24 h, the growth medium was removed, cells were accurately washed in Phosphate buffered saline (PBS; Sigma-Aldrich Corp., St. Louis, MO), and incubated in phenol red and serum-free medium. Cells were incubated (24 h) with IFN-γ (R&D Systems, Minneapolis, MN, USA; 500, 1000, 5000, 10000 IU/mL) and 10 ng/mL TNF-α (R&D Systems), alone or in combination. The concentration of TNF-α was selected in preliminary experiments to yield the highest responses. After 24 h, the supernatant was removed and kept frozen at −20 °C until CXCL10 assay [10,11].

To investigate the effect of PPAR-γ activators on IFN-γ-induced chemokine secretion, cells were stimulated (24 h) with IFN-γ (1000 IU/mL) and TNF-α (10 ng/mL) in the absence or presence of increasing concentrations (0, 1, 10, 20 μM) of the pure PPAR-γ agonists, rosiglitazone (RGZ, Glaxo, Welwyn, UK), or pioglitazone (Alexis Biochemicals, Lausen, Switzerland), and conditioned media were assayed by Enzyme-Linked ImmunoSorbent Assay (ELISA) for CXCL10 concentrations [10,11].

All experiments were repeated 3 times with the different cell preparations.

2.3. Cell viability test

The number of viable cells was evaluated by a viability and proliferation assay, based on the cleavage of tetrazolium salts added to the culture medium (Cell Proliferation Reagent WST-1; Roche, Mannheim, Germany) as previously reported [7–9].

2.4. ELISA for CXCL10

CXCL10 levels were measured in culture supernatants using commercially available kits (R&D Systems). The mean minimum detectable dose was 1.67 pg/mL for CXCL10; the intra- and inter-assay coefficients of variation were 3.0% and 6.9%. Samples were assayed in triplicate.

2.5. Apoptosis determination-Hoechst uptake and Annexin V binding

ANA cells were seeded in a 96-well microtiter plate at a concentration of 35000 cells/mL in a final volume of 100 μL in each well. Then, cultures were incubated for 48 h with PPAR-γ activator (10 or 20 μM, RGZ), in a humidified atmosphere (37 °C, 5% CO_2).

After 48 h of treatments, the cells were stained with 5 μg/mL of Hoechst 33342 for 10 min at 37 °C, and at the end of the incubation time, adherent cells were analyzed as previously reported [12].

The apoptosis index, (ratio between apoptotic and total cells) ×100, was calculated. Data were analyzed by one-way ANOVA with Newman–Keuls multiple comparisons test.

The apoptosis was confirmed by Annexin V binding assay as previously reported [12,13].

2.6. Nuclear extracts preparation, electrophoretic mobility shift assay (EMSA), immunoblotting

ANA and TFC were seeded in cell culture dishes at a concentration of 200000 cells/mL in a final volume of 10 mL. Then, cells were treated (1 h) with IFN-γ (1000 IU/mL) and TNF-α (10 ng/mL) in the absence or presence of RGZ 10 or 20 μM.

Nuclear extracts (nucleosol) were prepared, as reported previously [6].

EMSA and detection of the biotinylated sequence was performed as reported previously [13].

For immunoblotting, ANA cells were seeded in cell culture dishes at a concentration of 200000 cells/mL in a final volume of 10 mL. Then, cells were treated (24 h) with IFN-γ (1000 IU/mL) and TNF-α (10 ng/mL) in the absence or presence of RGZ 10 or 20 μM.

At the end of the treatments, the immunoblotting and immunodetection were performed as reported previously [6].

3. Results

3.1. CXCL10 secretion assay in ANA cells

In ANA cells, CXCL10 was undetectable in the supernatant in basal condition in preparations A–C, while a slight basal secretion was observed in preparations D–F, ranging from 3 to 11 pg/mL (Fig. 1).

IFN-γ dose-dependently induced the CXCL10 release (ANOVA, $p < 0.05$, *vs* basal), however the IFN-γ stimulation exerted a stronger effect in preparations B and D, while only a slight effect was shown in other preparations. Interestingly, the effect in preparations B and D was significantly higher than in TFC, while in the other preparations was weaker ($p < 0.01$, by ANOVA).

TNF-α alone in ANA cells had no effect on CXCL10, similarly to what observed in TFC.

The combination of IFN-γ + TNF-α had a significant synergistic effect on the CXCL10 secretion (Fig. 1). However, the synergistic effect of IFN-γ + TNF-α was significantly stronger on the CXCL10 secretion in ATC cells of preparations B and D, where IFN-γ alone exerted its stronger effect ($p < 0.001$, by ANOVA).

Treatment of ANA cells with RGZ, added at the time of IFN-γ + TNF-α stimulation, dose-dependently inhibited CXCL10 release (Fig. 1) in preparations A and E, stimulated it in preparation F (ANOVA, $p < 0.05$, *vs* IFN-γ + TNF-α), and had no significant effect in the others.

A similar effect was observed with pioglitazone (Fig. 1). Treatment of ANA cells with pioglitazone, added at the time of IFN-γ + TNF-α stimulation, dose-dependently inhibited CXCL10 release (Fig. 1) in preparation A, stimulated it in preparation F

Download English Version:

https://daneshyari.com/en/article/5898041

Download Persian Version:

https://daneshyari.com/article/5898041

Daneshyari.com