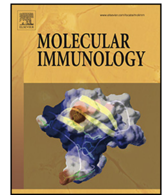
Molecular Immunology 68 (2015) 692–698

Contents lists available at ScienceDirect

Molecular Immunology

journal homepage: www.elsevier.com/locate/molimm



CRAM-A indicates IFN-γ-associated inflammatory response in breast cancer

Parisa Sarmadi [a], Gurcan Tunali [a], Guldal Esendagli-Yilmaz [b], Kerim Bora Yilmaz [c], Gunes Esendagli [a,*]

[a] Department of Basic Oncology, Hacettepe University Cancer Institute, Ankara, Turkey
[b] Department of Pathology, Medical Faculty, Gazi University, Ankara, Turkey
[c] Department of General Surgery, Diskapi Yildirim Beyazit Training and Research Hospital, Ankara, Turkey

ARTICLE INFO

Article history:
Received 25 June 2015
Received in revised form 24 October 2015
Accepted 26 October 2015
Available online 10 November 2015

Keywords:
CCRL2
Immune response
Breast cancer
Inflammation
Decoy receptor
Atypical chemokine receptor

ABSTRACT

Atypical chemokine receptors (ACKRs) function as endpoint regulators of chemokine gradients. These non-signaling receptors that are transiently expressed under inflammatory conditions have critical roles in the control or maintenance of immune responses. Alternatively, here, CCRL2 (ACKR5) expression was determined to be constitutive in breast cancer cells. Increased amount of CCRL2 was also found in breast tumor tissues with high immune infiltration. Its expression was upregulated in the presence of pro-inflammatory cytokines, IL-1β, TNF-α, IL-6, and especially IFN-γ. Moreover, an alternative transcript of CCRL2 gene, CRAM-A, was specifically expressed in a transient fashion under the influence of IFN-γ. CRAM-A expression was also positively correlated with the presence of IFN-γ mRNA in patient samples. CCRL2-associated chemotactic molecules, chemerin, CCL19 and CCL5, were also detected in cancer tissues and CCL5 mRNA level was correlated with that of CRAM-A and IFN-γ. Hence, in breast cancer, CRAM-A becomes specifically upregulated under inflammatory stimuli and may serve as a potential marker of immune response.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Non-signaling decoy receptors directly reduce the availability of ligands to bind functional receptors (Nathan, 2002; Mantovani et al., 2007). Accordingly, leukocyte recruitment is regulated by the atypical chemokine receptors (ACKRs) that can scavenge, internalize, transport and/or buffer chemotactic molecules (Nibbs and Graham, 2013). These seven transmembrane-spanning (7TM) receptors have been implicated in the processes basically associated with inflammation such as infections and in cancer (Mantovani et al., 2007; Nibbs and Graham, 2013).

CCRL2 has been provisionally designated as ACKR5 (Bachelerie et al., 2014). It concentrates chemerin (Zabel et al., 2008) and scavenges CCL5 and CCL19 chemokines, reportedly (Hartmann et al., 2008; Leick et al., 2010; Catusse et al., 2010). However, there are contradictions on ligand-scavenging functions of CCRL2 which might be due to interspecies differences, allele-specific expression and/or transcript variations (Forster and Sozzani, 2013; Yoshimura and Oppenheim, 2011). Human CCRL2 (HCR, CRAM) gene has two alternatively spliced transcripts, CRAM-A (CCRL2A) and CRAM-B (CCRL2B). CRAM-B protein is 12 amino acids shorter than that of encoded by CRAM-A variant (Forster and Sozzani, 2013). Similar to other ACKRs, CCRL2 expression is widely found in hematopoietic cells and transiently upregulated under inflammatory stimuli such as LPS and TNF-α (Zabel et al., 2008; Hartmann et al., 2008; Leick et al., 2010; Catusse et al., 2010; Forster and Sozzani, 2013; Yoshimura and Oppenheim, 2011). Accordingly, CCRL2 has been detected in inflammatory disorders and in certain cancers such as glioblastoma, B-cell chronic lymphocytic leukemia (B-CLL) and cervical carcinoma (Catusse et al., 2010; Yin et al., 2012; Hou et al., 2014).

In this study, breast cancer cells were determined to constitutively express CCRL2, predominantly the variant CRAM-B; however inflammatory stimuli differentially favored transcription of the

Abbreviations: 7TM, seven transmembrane-spanning; ACKR, atypical chemokine receptor; B-CLL, B-cell chronic lymphocytic leukemia; CCRL, C–C chemokine receptor-like; CRAM, chemokine receptor on activated macrophages; EGFP, enhanced green fluorescent protein; ER, estrogen receptor; IFN, interferon; IHC, immunohistochemistry; IL, interleukin; MFI, mean fluorescence intensity; LPS, lipopolysaccharide; ORF, open reading frame; PCR, polymerase chain reaction; PR, progesterone receptor; RT, reverse transcriptase; TNF, tumor necrosis factor.

* Corresponding author at: Department of Basic Oncology, Hacettepe University Cancer Institute, 06100 Sihhiye, Ankara, Turkey. Fax: +90 31 23 24 20 09.
E-mail addresses: gunes.esendagli@lycos.com, gunese@hacettepe.edu.tr (G. Esendagli).

http://dx.doi.org/10.1016/j.molimm.2015.10.019
0161-5890/© 2015 Elsevier Ltd. All rights reserved.

Table 1
Summary of the patient data.

Histopathology	Grade		HR status[a]	Age (mean ± SD)
Invasive ductal carcinoma (IDC)	1	$n=16$	16/16 ER^+	57 ± 11
	2	$n=24$	24/24 ER^+	51 ± 9
	3	$n=41$	25/41 ER^+	53 ± 10
			16/41 TN	53 ± 14
Normal breast tissue (reduction mammoplasty)	N/A	$n=5$	N/A	34 ± 11

[a] Hormone receptor (HR) status: Tumors with high estrogen receptor (ER) positivity indicated the luminal sub-type whereas the absence of ER, progesterone receptor (PR) and Her2 overexpression (i.e., triple-negative (TN) breast cancers) was used as an indicator of basal-like sub-type. (N/A, not applicable).

variant CRAM-A. Particularly, IFN-γ was the major cytokine that induced the expression of CRAM-A. Thus, the presence of CRAM-A might serve as a putative indicator of IFN-γ mediated immune responses in breast cancer.

2. Materials and methods

2.1. Patients and tissue specimens

Experimental procedures were approved by the local ethics committee at Hacettepe University and at Diskapi Yildirim Beyazit Training and Research Hospital. Specimens obtained from 21 patients undergoing surgical resection were protected in RNAlater solution (Ambion, Life Technologies, Carlsbad, CA, USA). In addition to these freshly collected specimens, formalin-fixed, paraffin-embedded total 60 tumor or 5 normal breast tissue samples were obtained from pathology archives. Tumor grading was based on the World Health Organization criteria (Table 1). For this purpose, tissue sections were histopathologically evaluated under light microscopy following conventional hematoxylin–eosin and immunohistochemistry (IHC) staining of ER, PR and Her2 molecules. Individual specimens were also subjected to semi-quantitative scoring (+, sparse/focal; ++, diffuse/multifocal; +++, diffuse/encompassing) for immune infiltration.

2.2. Cell culture

Luminal (SK-BR-3, MCF-7, BT-474, T47-D, and ZR-75-1) and triple-negative (MDA-MB-468, MDA-MB-231, HCC38, and MCF-12A) breast cell lines and HEK293T cells were previously obtained (ATCC, LGC Promochem, Rockville, MD, USA). The cells were cultured in appropriate media and under standard conditions as instructed by the provider. In certain experimental setups, breast cancer cells (10^5/ml) were stimulated with recombinant IL-1β (400 pg/ml), TNF-α (1.6 ng/ml), IFN-γ (150 ng/ml or 300 ng/ml) or IL-6 (20 ng/ml) (R&D, Minneapolis, MN, USA) cytokines.

2.3. Immunohistochemistry (IHC)

Immunohistochemistry was performed on 4 μm-thick tissue sections following deparaffinization, hydration, blocking of endogenous peroxidase activity, antigen retrieval (microwave treatment in pH 6.0 citrate buffer for 20 min), several washing steps, and incubation with non-immune protein blocking serum for 10 min. The slides were covered with rabbit anti-human CCRL2 primary antibody (dilution 1/100; 1 h incubation at room temperature) (HPA043238, Prestige Antibodies®, Atlas Antibodies, Sigma, St. Louis, MO, USA). Antibody binding was determined by polyvalent secondary antibodies using a biotin/streptavidin/horseradish peroxidase (HRP)/diaminobenzidine (DAB) tetrahydrochloride substrate detection system (multi-species ultra-streptavidin detection system-HRP, Lab Vision, Fremont, CA, USA). The slides were counter stained with Harris' hematoxylin. CCRL2 staining was subjected to semiquantitative evaluation by pathology specialists according to intensity (−, no; +, weak; ++, moderate; +++, strong) and pattern which is a regular practice also for diagnostic evaluation of IHC results. Representative micrographs with different staining intensities are shown in Supplementary Fig. 1.

2.4. Flow cytometry

Cells were labeled with anti-human-CCRL2-APC (clone 152254, recognizing both CRAM-A and CRAM-B isoforms) (R&D) monoclonal antibody. The percentage of positive cells was calculated in comparison to the staining with isotype-matched antibody. For the analysis of enhanced green fluorescent protein (EGFP), untransfected cells were used for autofluorescence correction. Mean fluorescence intensity (MFI) values were also determined. Analyses were done on a FACSAria II cell sorter (Becton Dickinson, San Jose, CA, USA).

2.5. Reverse transcription–polymerase chain reaction (RT–PCR)

Total RNA was obtained either from breast cancer cell lines or from patient tumor samples homogenized in buffer RLT (QIAamp RNA Mini Kit, QIAGEN, Maryland, MD, USA) and residual DNA was eliminated (DNA-free RNA Kit, Zymo Research, Irvine, CA, USA). cDNA was synthesized from 2 μg or 0.7 μg mRNA by using oligo(dT) primers (RevertAid™ First Strand cDNA Synthesis Kit, Fermentas, Vilnius, Lithuania). The primer sequences and annealing temperatures used in PCR reactions are given in Supplementary Table 1. Schematic presentation of the CCRL2 transcripts and primer binding sites are supplied in Supplementary Fig. 2. PCR products were separated on 2% agarose gel electrophoresis, stained with ethidium bromide, and documented under UV light. Intensities of the bands, which were acquired at an amplification cycle empirically determined to coincide with logarithmic phase (18–27 cycles) of the reaction, were measured for semiquantitative evaluation of gene expression (Kodak Gel Logic 1500 Imaging System, Carestream Health, Rochester, NY, USA).

Real-time RT–PCR experiments were performed with FastStart DNA Master SYBR Green I (Roche Diagnostics, Mannheim, Germany). PCRs contained 0.125 μM primer oligonucleotides and 3.5 mM $MgCl_2$ and carried out on a Rotor-Gene 6000™ cycler (Corbett Research, Sydney, Australia). Data were obtained with cDNAs synthesized from two independent experiments and PCRs were run in duplicates. The relative difference in gene expression was calculated with comparative Ct ($2^{-\Delta\Delta Ct}$) method where amplification data obtained from the gene of interest were normalized to β-actin housekeeping gene expression and then with the data from control (untreated) samples. Hence, the $2^{-\Delta\Delta Ct} = 1$ value indicates equal level of gene expression between the control and other groups compared (Pfaffl, 2001).

2.6. Western blot

Control and IFN-γ-treated breast cancer cells (2×10^6) were lysed in EBC lysis buffer (50 mM Tris–HCl pH 7.4 (Applichem, Darmstadt, Germany), 120 mM NaCl (Merck, Darmstadt, Germany), 1 mM $Na_4P_2O_7$, 0.5% NP-40, 2 mM EDTA, 2 mM EGTA (Sigma)) con-

Download English Version:

https://daneshyari.com/en/article/2830687

Download Persian Version:

https://daneshyari.com/article/2830687

Daneshyari.com