Biochemical and Biophysical Research Communications 504 (2018) 270–276

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Protease-activated receptor 2 (PAR2) upregulates granulocyte colony stimulating factor (G-CSF) expression in breast cancer cells

Érika Carvalho [a], Vitor Hugo de Almeida [a], Araci M.R. Rondon [a], Patricia A. Possik [b], João P.B. Viola [b], Robson Q. Monteiro [a, *]

[a] Institute of Medical Biochemistry Leopoldo de Meis, Federal University of Rio de Janeiro, RJ, Brazil
[b] Program of Cellular Biology, Brazilian National Cancer Institute (INCA), Rio de Janeiro, RJ, Brazil

ARTICLE INFO

Article history:
Received 19 August 2018
Accepted 27 August 2018
Available online 30 August 2018

Keywords:
Protease-activated receptor
Granulocyte colony stimulating factor
Breast cancer
TCGA
ENMD-1068

ABSTRACT

Protease-activated receptor 2 (PAR2) is a G-protein coupled receptor which is activated upon cleavage of its N-terminal region. PAR2 has been associated with many aspects regarding tumor progression, such as the production of pro-tumoral cytokines. Granulocyte colony-stimulating factor (G-CSF) is a cytokine essential to neutrophil production and maturation, and it is often overexpressed in tumors. In this study, we evaluated the ability of PAR2 to modulate G-CSF expression. *PAR2* and *G-CSF* were significantly more expressed in metastatic (4T1 and MDA-MB-231) as compared to non-metastatic (67NR and MCF7) breast cancer cell lines. In addition, PAR2 stimulation by a synthetic agonist peptide significantly increased *G-CSF* gene expression in the metastatic cell lines. Knockdown of PAR2 in 4T1 cells decreased G-CSF expression and secretion. In addition, treatment of 4T1 with the commercial PAR2 antagonist, ENMD-1068, significantly decreased *G-CSF* expression. cBioPortal analyses of the TCGA database showed a significant co-occurrence of *G-CSF* and *PAR2* gene overexpression in breast cancer samples. In conclusion, our data suggest that PAR2 contributes to G-CSF expression in breast cancer cells, possibly favoring tumor progression.

1. Introduction

Protease-activated receptors (PARs) are a distinct class of G protein-coupled receptors activated by proteolysis [1]. Activation of all members is initiated by N-terminal cleavage of the receptors, which exposes a unique amino acid sequence called tethered ligand. This ligand interacts with the second extracellular loop of the protein inducing conformational changes and activation of intracellular signaling pathways mainly by G proteins. PARs, more specifically PAR1 and PAR2, have been associated with several steps of cancer development and progression [2–4]. The predominant activators of PAR2 are trypsin, tryptase, binary complex tissue factor (TF)/coagulation factor VIIa (FVIIa), and ternary complex TF/FVIIa/FXa [4]. PAR2 activation promotes tumor growth, chemoresistance, migration, and angiogenesis in different cancer models [5–8]. Remarkably, overexpression of PAR2 has been associated with worse prognosis in breast cancer patients [9].

Granulocyte colony-stimulating factor (G-CSF) is a small cytokine that regulates neutrophil production in homeostasis and in response to infections. G-CSF induces proliferation of non-committed hematopoietic stem cells and granulocytic lineage due to tissue-specific gene expression of its receptor, G-CSFR [10]. Both G-CSF and G-CSFR are overexpressed in different tumor types [11–13] and several lines of evidence suggest that G-CSF supports tumor progression [14]. G-CSF is highly expressed in human breast cancer samples from the triple-negative aggressive subtype and correlates with poor overall survival [15]. Indeed, animal models demonstrate that G-CSF plays a role in tumor metastasis [16,17].

In the current study, we demonstrate that PAR2 modulates G-CSF expression in human and murine breast cancer cell lines *in vitro*. In addition, cBioPortal analyses of The Cancer Genome Atlas (TCGA) database showed significant co-occurrence of G-CSF and PAR2 overexpression in human breast cancer samples. Taken together, these results suggest that G-CSF expression might be stimulated by PAR2 signaling in tumor cells, which may be relevant for tumor progression and clinical management of patients.

* Corresponding author. IBqM/CCS/UFRJ, Avenida Carlos Chagas Filho 373 (Bloco H, segundo andar, sala 08), Cidade Universitária, Ilha do Fundão, Rio de Janeiro, 21941-599, Brazil.
E-mail address: robsonqm@bioqmed.ufrj.br (R.Q. Monteiro).

https://doi.org/10.1016/j.bbrc.2018.08.169
0006-291X/

2. Material and methods

2.1. Reagents

Human (SLIGKV-NH2) and mouse (SLIGRL-NH2) PAR2 agonist peptides (PAR2-AP) were synthesized by Biosynthesis Inc. (Lewisville, TX, USA). ENMD-1068 was purchased from Abcam (MA, USA).

2.2. Cell culture

The cells lines 4T1 (metastatic) and 67NR (non-metastatic), originated from spontaneous breast tumors in Balb/c mouse [18] were purchased from Karmanos Cancer Institute (Detroit, MI, USA). 4T1 cells were grown in RPMI 1640 medium (Gibco®; Life Technologies, MA, USA) supplemented with 10% fetal bovine serum (FBS). 67NR, as well as the human breast cancer cell lines MDA-MB-231 (metastatic) and MCF7 (non-metastatic), were grown in Dulbecco's Modified Eagle Medium (DMEM, Gibco®; Life Technologies, MA, USA) supplemented with 10% FBS. HEK293FT cell line was used for lentivirus particles packing and maintained in DMEM supplemented with 10% FBS. All supplements used were purchased from Gibco® Life Technologies (MA, USA).

2.3. Plasmid construction

The guideRNA (gRNA) for mouse *PAR2* gene were designed using the online platforms: CRISPR Design (http://crispr.mit.edu/) [19] and CRISPRscan (http://www.crisprscan.org/) [20]. The following oligonucleotides were synthesized for cloning: F: CACCGTATCACCCTTCTG GCGGCCT, R: AAACAGGCCGCCAGAAGGGTGATAC. Oligonucleotides were annealed to form double-stranded DNA in a 10 μL reaction containing: 1 μL of each primer (IDT, IA, USA), 1 μL 10× ligase buffer (New England Biolabs, MA, USA), and 7 μL water. The reaction was incubated for 30 min at 37 °C followed by 5 min at 95 °C, reducing 5 °C/min until 25 °C. Annealed oligonucleotides were ligated to BsmBI (Thermo Fisher Scientific, MA, USA) digested lentiCRISPRv2 (Addgene: #52961) by mixing: 1 μL annealed primers (diluted 1:100), 2 μL 10× T4 ligase buffer (New England Biolabs, MA, USA), 2 μL T4 DNA ligase and 15 μL of water and incubating overnight at 16 °C. Electrocompetent *Escherichia coli* XL1-Blue bacteria were transformed by electroporation. Clones were expanded and constructs were confirmed by DNA sequencing.

2.4. Production of recombinant lentiviruses and infection of target cells

HEK293FT cells (ATCC – 4×10^6 cells/plate) were plated on 10 cm diameter plates and transiently transfected by calcium phosphate precipitation on the next day. For that, 3 μg of each accessory plasmid (pRSV rev, pMDLg/pRRE and pHCMV-G) and 8 μg of the control or gRNA plasmids (empty lentiCRISPRv2 and lentiCRISPRv2-PAR2gRNA) were added to 500 μL of a 0,25 M $CaCl_2$ solution. Then, 500 μL of HEPES Buffer Saline (HBS) 2× (280 mM NaCl; 10 mM KCl; 1.5 mM Na_2HPO_4; 12 mM dextrose; 50 mM HEPES; pH7,1) was added to the transfection mixture while vortexing. Cells were incubated in media containing 25 μM chloroquine and the transfection mixture (1 mL) for 6 h. Forty-eight hours after transfection, lentivirus-containing supernatants were collected, filtered and stored at −80 °C. For lentiviral transductions, 4×10^5 of 4T1 cells were plated on 10 cm diameter plates and, on the next day, incubated with 1:2 (total: 8 ml) diluted virus supplemented with 8 μg/mL polybrene. After 24 h, cells were cultivated for 15 days in media containing 15 μg/mL puromycin to select for cells with stable integration of the expression cassette. A pooled, polyclonal cell

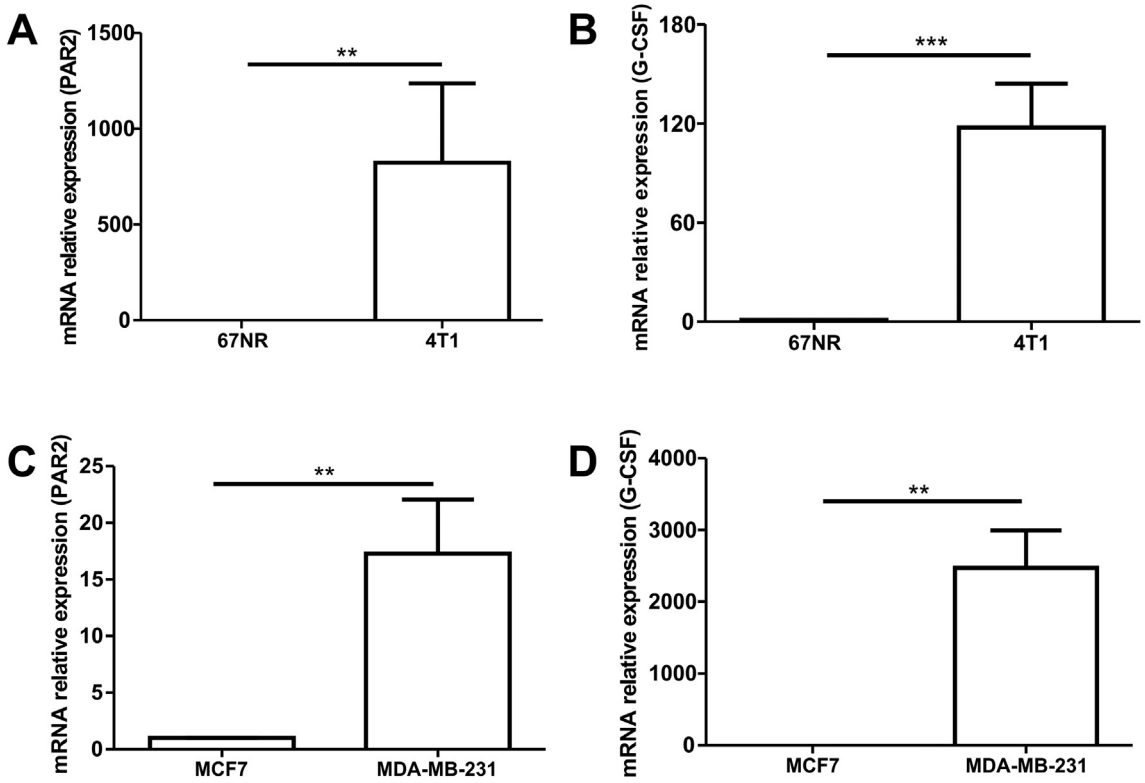

Fig. 1. PAR2 and G-CSF are upregulated in metastatic compared to non-metastatic breast cancer cell lines. Relative expression of PAR2 and G-CSF was evaluated by qPCR on 67NR and 4T1 cell lines (A, B) and MCF7 and MDA-MB-231 cell lines (C, D). Bar graphs show gene expression in metastatic cell lines relative to non-metastatic. Mouse β-actin and human TBP were used as reference genes. Values represent mean + SD of at least three independent experiments. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

population likely carrying different genetic editing alterations was selected and partial PAR2 knockout was validated by western blotting. This cell line was designated 4T1-CRISPR. 4T1 cells transduced with empty vector were designated 4T1-Vector.

2.5. RNA isolation, reverse transcription and qPCR

For analysis of basal gene expression levels, cells were seeded on 6-well plates and assayed after 24 h (1×10^5 cells/well). For agonist stimulation experiments, 4T1 and MDA-MB-231 cells were plated on 6-well plates (1×10^5 cells/well). The next day, cells were starved for 16 h and subsequently stimulated with either the mouse (100 μM) or the human (50 μM) PAR2-AP for 90 min. For antagonist experiment, 4T1 cells were plated on 6-well plates (1×10^5 cells/well). The next day, cells were starved for 16 h and treated with ENMD-1068 (0.25 mM, 2.5 h), PAR2-AP (100 μM, 1.5 h), or ENMD-1068 + PAR2-AP (pre-treatment with 0.25 mM ENMD-1068 for 1 h and stimulation with 100 μM PAR2-AP for 1.5 h). RNA was isolated using Trizol reagent (Thermo Fisher Scientific, MA, USA) following the manufacturer's instructions. Total RNA (1 μg) was treated with RNase-free DNase I (Thermo Fisher Scientific, MA, USA) and reverse-transcribed into cDNA using SuperScript II (Thermo Fisher Scientific, MA, USA) following the manufacturer's protocol. cDNA was diluted ten times and added to a 10 μL of final volume PCR reaction. qPCR was performed using SYBR green PCR master mix (Thermo Fisher Scientific, MA, USA) or Taqman master mix (Thermo Fisher Scientific, MA, USA). TaqMan gene expression assays: mouse *beta-actin/ACTB* (Mm00607939_s1, housekeeping); mouse *G-CSF/CSF3* (Mm00438334_m1); mouse *F2RL1/PAR2* (Mm00433160_m1); mouse *CXCL1* (Mm04207460_m1). Primers sequence: human *TBP* (housekeeping), F: ACAACAGCCTGCCACCTTAC, R: GTTCTGAATAGGCTGTGGGG; human *G-CSF*, F: AAGGGATCTCCCCCGAGTTG, R: CAGATGGTGGTGGCAAAGTC. Reactions were standardized for amplification efficiency between 90 and 110%. All assays were performed at ABI 7500 equipment (Thermo Fisher Scientific, MA, USA). The delta-delta Ct method was used to determine the relative levels of mRNA expression between samples.

2.6. Western blot

For analysis of basal expression, 4T1-Vector and 4T1-CRISPR cells were cultivated for 24 h prior to protein extraction. For agonist stimulation experiments, 4T1-Vector and 4T1-CRISPR cells were plated on 10 cm plates (1×10^6 cells/plate). The next day, cells were starved for 4h and stimulated with mouse (10 μM) PAR2-AP for 10 min.

Protein extracts were prepared with RIPA Buffer 2× supplemented with protease and phosphatase inhibitors. Protein quantitation was performed using *DC Protein Assay kit* (BioRad, CA, USA). Proteins (60 μg) were resolved by SDS-PAGE and transferred to nitrocellulose membranes (GE Healthcare Life Sciences, IL, USA) with *Trans-Blot Semi-Dry electrophoretic transfer cell* (BioRad, CA, USA). Membranes were blocked with Tris-buffered saline (TBS) containing 5% milk or bovine serum albumin for 1 h at room temperature. Membranes were then probed with primary antibodies overnight at 4 °C followed by secondary antibodies conjugated to horseradish peroxidase for 1h at room temperature. Primary antibodies used were as follows: PAR2 (1:1000 – ab180953 – Abcam, MA, USA); β-actin (1:1000 – #8457 – Cell Signaling Technology, MA, USA); p-ERK (1:1000 – #9101 – Cell Signaling Technology, MA, USA); α-tubulin (1:1000 – #2144 – Cell Signaling Technology, MA, USA). Membranes were developed with SuperSignalTM West Pico Chemiluminescent Substrate (Thermo Fisher Scientific, MA, USA) and results were detected in *Kodak BioMax Light Film* (Kodak, NY, USA).

2.7. Cell proliferation assay by crystal violet staining

4T1, 4T1-Vector and 4T1-CRISPR cells were plated in triplicate on 96-well microtiter plates (5×10^2 cells/well). Cells were fixed with ethanol and stained with 0.05% crystal violet in 20% ethanol at different time points. For relative quantification of cell number at different time points and conditions, crystal violet staining was extracted with methanol and the optical density was read at 595 nm on a spectrophotometer.

2.8. ELISA

4T1, 4T1-Vector and 4T1-CRISPR cells (1×10^5) were cultivated in 6-wells plates for 48 h. Cell-free supernatants were collected and G-CSF protein levels were quantified by *Mouse G-CSF DuoSet Elisa kit* (DY414; R & D Systems; Minneapolis, MN, USA), according to manufacturer's instructions.

2.9. Data analysis of breast cancer samples using cBioPortal

Gene expression data from Breast Invasive Carcinoma available at the TCGA database (TCGA, PanCancer Atlas) was assessed using

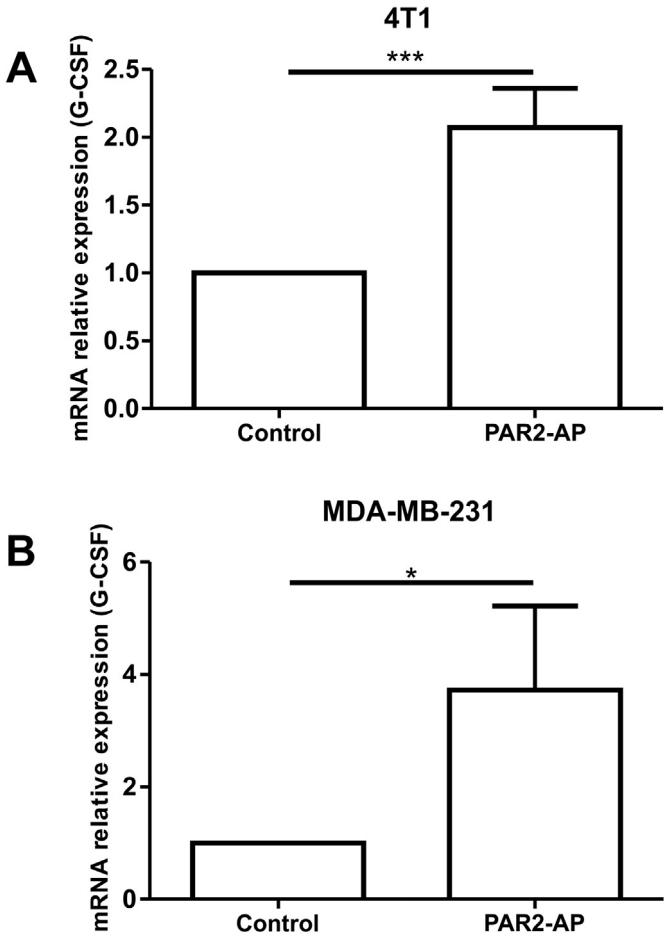

Fig. 2. PAR2 activation induces G-CSF expression in metastatic breast cancer cell lines. 4T1 and MDA-MB-231 cell lines were starved for 16h and, stimulated with either the mouse (100 μM) or the human (50 μM) PAR2-AP for 90 min (A) G-CSF relative expression in control and stimulated 4T1 cells evaluated by qPCR. (B) G-CSF relative expression in control and stimulated MDA-MB-231 cells evaluated by qPCR. Bar graphs show gene expression in PAR2-AP stimulated cell line relative to non-stimulated cell lines. Mouse β-actin and human TBP were used as reference genes. Values represent mean + SD of at least three independent experiments. *$p < 0.05$; ***$p < 0.001$.

the cBioPortal (http://cbioportal.org) to investigate the tendency of co-occurrence of *CSF3* (G-CSF) and *F2R* (PAR1), *F2RL1* (PAR2), *F2RL2* (PAR3) and *F2RL3* (PAR4) overexpression [21,22].

2.10. Statistical analysis

Bar graphs represent mean of at least three independent experiments and standard deviation. After gene expression levels normalization, Shapiro-Wilk (1965) and Levene (1960) tests were conducted. Then, Student's *t*-test or One-Way ANOVA was applied considering confidence interval of 95% ($P < 0.05$). Statistical analyses were performed using GraphPad Prism 6.0 (GraphPad Software Incorporated, San Diego, CA, USA).

3. Results

3.1. PAR2 and G-CSF are overexpressed in metastatic compared to non-metastatic breast cancer cell lines

Increased PAR2 and G-CSF expression have been independently correlated with cancer aggressiveness. Thus, we evaluated PAR2 and G-CSF gene expression in mouse (67NR and 4T1) and human (MCF-7 and MDA-MB-231) breast carcinoma cell lines. PAR2 expression levels were ~800-fold higher in 4T1 than in 67NR cell line (Fig. 1A) while the MDA-MB-231 cell line showed 17-fold higher expression levels in comparison to MCF-7 (Fig. 1C). Similarly, G-CSF expression levels were significantly higher in the metastatic cell lines: 4T1 and MDA-MB-231 showed 120- and 2500-fold higher expression levels compared to their respective non-metastatic counterparts (Fig. 1B and D). In conclusion, relative gene expression analysis revealed that PAR2 and G-CSF levels were significantly higher in the metastatic cancer cell lines (4T1 and MDA-MB-231) compared to the non-metastatic ones (67NR and MCF-7) (Fig. 1).

3.2. PAR2 activation induces G-CSF expression in the metastatic cell lines

Next, we evaluated whether PAR2 activation was sufficient to induce G-CSF expression. Serum-deprived 4T1 and MDA-MB-231 cell lines were treated with synthetic agonist peptides for PAR2 (PAR2-AP). The 4T1 stimulation caused a 2-fold increase in G-CSF gene expression (Fig. 2A). PAR2-AP stimulation of MDA-MB-231 increased G-CSF gene expression 4-fold compared to untreated control (Fig. 2B).

3.3. Partial knockout of PAR2 in the 4T1 cell line

To confirm whether PAR2 expression and, consequently, PAR2 receptor signaling plays a role in G-CSF expression, a partial knockout of PAR2 in 4T1 cells was generated using the CRISPR/Cas9 approach. A guideRNA specific for *PAR2* gene sequence was designed and cloned into a lentiviral vector carrying the endonuclease Cas9 DNA sequence. Genetic editing of the *PAR2* open reading frame generated a decrease in PAR2 protein expression levels in 4T1 cells transduced with gRNA specific to PAR2 (4T1-CRISPR) compared to cells transduced with the empty vector (4T1-VECTOR) (Fig. 3A). Because here we use a pooled population of genetically edited cells, we achieved the partial PAR2 knockout, indicating that part of the edited cells harbor frameshift alterations and protein ablation. This effect was sufficient to observe the functional consequences of PAR2 down modulation in metastatic

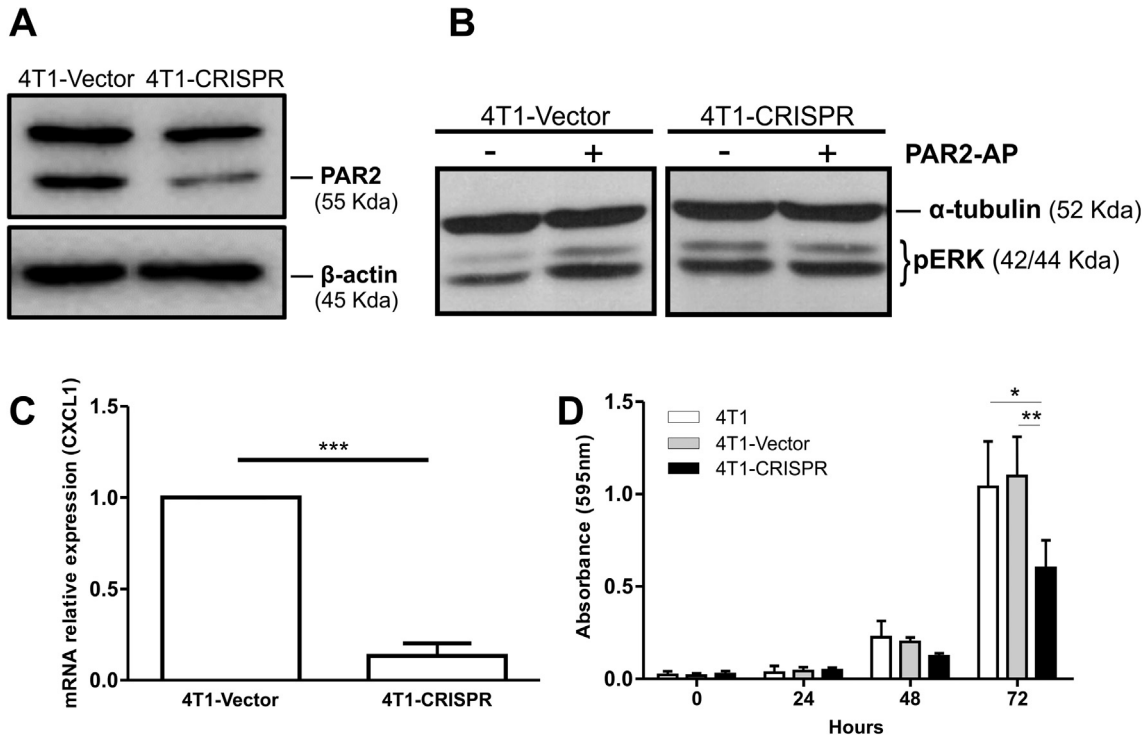

Fig. 3. PAR2 pooled knockout validation in the 4T1 cell line. (A) Western blot analysis of PAR2 expression in 4T1-Vector and 4T1-CRISPR cells. β-actin was used as loading control. (B) Western blot analysis of phospho-ERK levels in 4T1-Vector and 4T1-CRISPR cells with and without stimulation with PAR2-AP (10 μM) for 10 min α-tubulin was used as loading control. (C) CXCL1 relative expression in wild-type 4T1, 4T1-Vector and 4T1-CRISPR cells evaluated by qPCR. Bar graphs show gene expression in 4T1-Vector and 4T1-CRISPR cells lines relative to wild-type 4T1 cells. Mouse β-actin was used as a reference gene. (D) Proliferation kinetic assay by crystal violet staining. Cells (5×10^2) were plated and stained every 24 h until 72 h. Values represent mean + SD of at least three independent experiments. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

breast cancer cells. 4T1-CRISPR cells showed reduced ERK phosphorylation in response to PAR2-AP treatment (Fig. 3B). 4T1-CRISPR expressed lower levels of CXCL1, a known PAR2 target gene (Fig. 3C). Moreover, 4T1-VECTOR and wild-type 4T1 cells proliferated two times faster than 4T1-CRISPR cells (Fig. 3D).

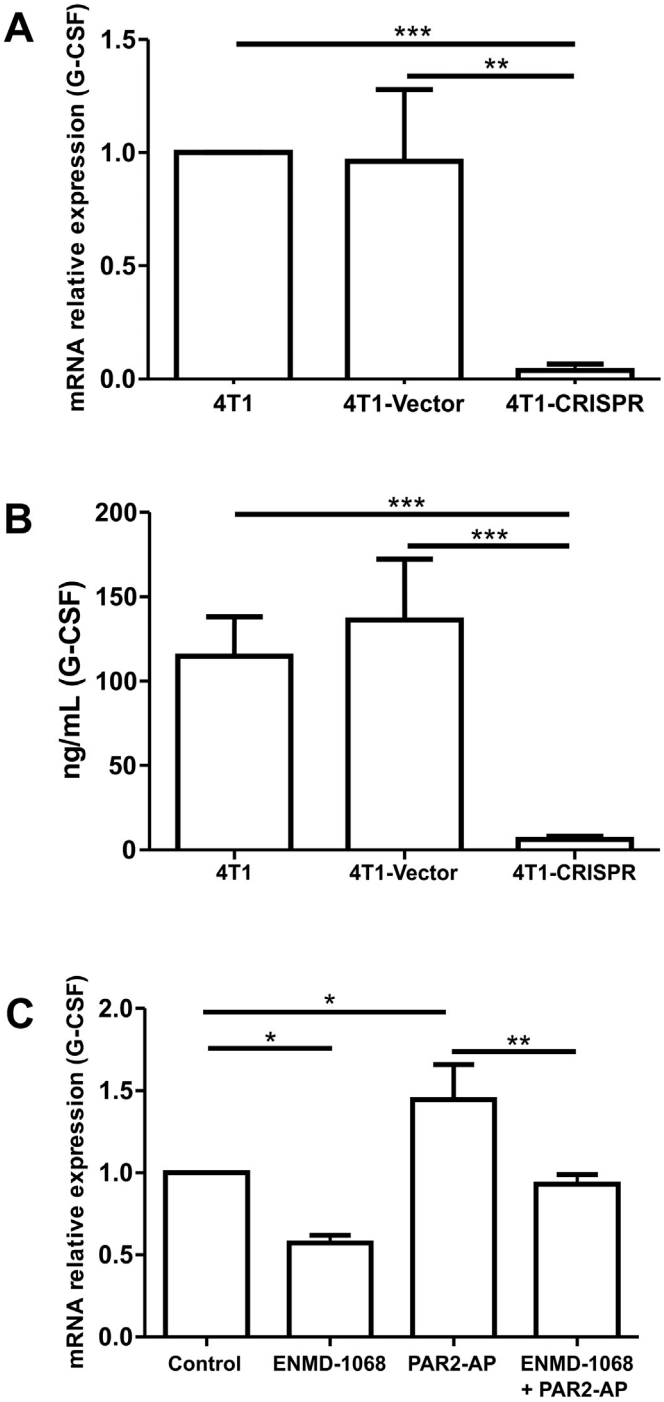

Fig. 4. 4T1-CRISPR cells express and secrete lower levels of G-CSF. (A) G-CSF relative expression in wild-type 4T1, 4T1-Vector and 4T1-CRISPR cells evaluated by qPCR. Bar graphs show the gene expression of 4T1-Vector and 4T1-CRISPR cell lines relative to wild-type 4T1 cells. (B) Wild-type 4T1, 4T1-Vector and 4T1-CRISPR cells were plated and cell-free supernatant was assessed for G-CSF protein levels by ELISA after 48h. (C) G-CSF relative expression in 4T1 cells treated with ENMD-1068 (0,25 mM, 2.5 h), PAR2-AP (100 μM, 1.5 h), and ENMD-1068 + PAR2-AP (pre-treatment with 0,25 mM ENMD-1068 for 1h and stimulation with 100 μM PAR2-AP for 1.5 h). Mouse β-actin was used as a reference gene. Values represent mean + SD of at least three independent experiments. *p < 0.05; **p < 0.01; ***p < 0.001.

3.4. Genetic or pharmacological inhibition of PAR2 reduces G-CSF expression in 4T1 cells

To evaluate the impact of the genetic inhibition of PAR2 in 4T1 cells, we further evaluated the gene expression and the secretion of G-CSF in 4T1, 4T1-VECTOR and 4T1-CRISPR cells. Quantitative PCR showed that 4T1-CRISPR cells expressed 30-fold fewer G-CSF levels than wild-type 4T1 and 4T1-VECTOR cells (Fig. 4A). Consistently, ELISA assays showed that secretion of G-CSF by 4T1-CRISPR cells, as compared to wild-type 4T1 and 4T1-VECTOR, was reduced by ~20-fold (Fig. 4B). Finally, treatment of 4T1 cells with the commercial PAR2 agonist ENMD-1068 decreased G-CSF gene expression by ~45% even after stimulation with the PAR2 agonist PAR2-AP (Fig. 4C).

3.5. Co-occurrence of G-CSF and PAR2 overexpression in breast carcinoma

To address whether our observations on cell lines may be translated to a clinical context, we investigated the co-occurrence of overexpression of G-CSF and PAR in samples of human invasive breast carcinoma available at the TCGA database (PanCancer Atlas) by using cBioPortal (33, 34). As shown in Table 1, we found that G-CSF and PAR2 overexpression frequently co-occur ($P < 0.001$) in breast cancer samples. In contrast, no significant co-occurrence between G-CSF and PAR1, PAR3 or PAR4 overexpression was observed (Table 1).

4. Discussion

The expression PAR2 and G-CSF have been independently associated with metastasis and/or poor prognosis in breast cancer [9,15]. In the present study, we demonstrate that PAR2 and G-CSF are overexpressed in metastatic cell lines as compared to non-metastatic counterparts. PAR2 activation increases G-CSF expression in human and murine breast cancer cells. In addition, cBioPortal analyses of The Cancer Genome Atlas (TCGA) gene expression database showed significant co-occurrence of G-CSF and PAR2 overexpression in human breast cancer samples.

Tumor models relying on increased G-CSF levels in the bloodstream exhibit neutrophilia [23] and the release of immature cells from bone marrow [10]. The role of neutrophil in cancer metastasis has been extensively studied and recent evidence implicate G-CSF in this process [14]. G-CSF has been shown to prime pre-metastatic sites through expansion and recruitment of neutrophils [16]. In addition, G-CSF neutralization or neutrophil depletion reduces metastasis formation [16,17]. On the other hand, injection of 67NR non-metastatic cells in Balb/C mice pre-treated with G-CSF lead to lung metastasis formation [16]. In addition, G-CSF appears to be essential for induction of myeloid-derived suppressor cells (MDSCs), a heterogeneous population of monocytes and granulocytes that suppress the immune system and is related to tumor growth [24,25].

Neutrophil-derived elastase may cleave and activate PAR2 [26]. Thus, it is reasonable to speculate the existence of a positive feedback loop in the tumor microenvironment in which G-CSF recruits

Table 1
Co-occurrences between the indicated genes (C-BIOPORTAL/TCGA Analysis).

Gene A	Gene B	p-Value	Log Odds Ratio	Tendency
CSF3 (G-CSF)	*F2R* (PAR1)	0.506	< −3	Mutual exclusivity
	F2RL1 (PAR2)	<0.001**	2.900	Co-occurrence**
	F2RL2 (PAR3)	0.537	0.336	Co-occurrence
	F2RL3 (PAR4)	0.489	0.481	Co-occurrence

neutrophils to produce elastase, activating PAR2, and leading to more G-CSF secretion. In this context, MDA-MB-231 express and secrete trypsin, which may continuously stimulate PAR2 [27]. Although this aspect was not evaluated in this work, it may explain the discrepancy between 4T1 and MDA-MB-231 regarding the levels of G-CSF induction by the PAR2 agonist peptide.

A number of PAR2 antagonists have been identified, some presenting significant antitumor properties [28–32]. ENMD-1068 is the only commercial PAR2 antagonist available. In epithelial ovarian cancer cells, ENMD-1068 was shown to abolish the migration and release of VEGF-A [31]. Treatment of cancer cell lines with I-191 reduced *in vitro* migration and cytokine expression [32]. Taken together, we propose that PAR2 inhibition could decrease G-CSF production affecting tumor progression in several ways. Therefore, it remains to be determined whether the PAR2/G-CSF signaling axis is relevant *in vivo*.

Conflicts of interest

All authors have no conflicts of interest to disclose.

Acknowledgements

Authors thank Dr T.M. Tilli for helpful discussions. This work was supported by the Brazilian National Council for Scientific and Technological Development (CNPq) [grant number 308797/2014-0]; The State of Rio de Janeiro Research Foundation (FAPERJ) [grant number E-26/203.028/2015], and the Coordination for the Improvement of Higher Education Personnel (CAPES) [grant number 23038.004147/ 2010-27].

Transparency document

Transparency document related to this article can be found online at https://doi.org/10.1016/j.bbrc.2018.08.169.

References

[1] S.R. Coughlin, Protease-activated receptors in hemostasis, thrombosis and vascular biology, J. Thromb. Haemostasis 3 (2005) 1800–1814. https://doi.org/10.1111/j.1538-7836.2005.01377.x.
[2] L.G. Lima, R.Q. Monteiro, Activation of blood coagulation in cancer: implications for tumour progression, Biosci. Rep. 33 (2013), e00064. https://doi.org/10.1042/BSR20130057.
[3] W. Ruf, J. Disse, T.C. Carneiro-Lobo, N. Yokota, F. Schaffner, Tissue factor and cell signalling in cancer progression and thrombosis, J. Thromb. Haemostasis 9 (2011) 306–315. https://doi.org/10.1111/j.1538-7836.2011.04318.x.
[4] M.Z. Wojtukiewicz, D. Hempel, E. Sierko, S.C. Tucker, K.V. Honn, Protease-activated receptors (PARs)–biology and role in cancer invasion and metastasis, Canc. Metastasis Rev. 34 (2015) 775–796. https://doi.org/10.1007/s10555-015-9599-4.
[5] G.M. Hjortoe, L.C. Petersen, T. Albrektsen, B.B. Sorensen, P.L. Norby, S.K. Mandal, U.R. Pendurthi, L.V. Rao, Tissue factor-factor VIIa-specific up-regulation of IL-8 expression in MDA-MB-231 cells is mediated by PAR-2 and results in increased cell migration, Blood 103 (2004) 3029–3037. https://doi.org/10.1182/blood-2003-10-3417.
[6] H.H. Versteeg, F. Schaffner, M. Kerver, L.G. Ellies, P. Andrade-Gordon, B.M. Mueller, W. Ruf, Protease-activated receptor (PAR) 2, but not PAR1, signaling promotes the development of mammary adenocarcinoma in polyoma middle T mice, Canc. Res. 68 (2008) 7219–7227. https://doi.org/10.1158/0008-5472.CAN-08-0419.
[7] S. Su, Y. Li, Y. Luo, Y. Sheng, Y. Su, R.N. Padia, Z.K. Pan, Z. Dong, S. Huang, Proteinase-activated receptor 2 expression in breast cancer and its role in breast cancer cell migration, Oncogene 28 (2009) 3047–3057. https://doi.org/10.1038/onc.2009.163.
[8] V. Hugo de Almeida, I.D.S. Guimarães, L.R. Almendra, A.M.R. Rondon, T.M. Tilli, A.C. de Melo, C. Sternberg, R.Q. Monteiro, Positive crosstalk between EGFR and the TF-PAR2 pathway mediates resistance to cisplatin and poor survival in cervical cancer, Oncotarget 9 (2018) 30594–30609. https://doi.org/10.18632/oncotarget.25748.
[9] L. Rydén, D. Grabau, F. Schaffner, P.E. Jönsson, W. Ruf, M. Belting, Evidence for tissue factor phosphorylation and its correlation with protease-activated receptor expression and the prognosis of primary breast cancer, Int. J. Canc. 126 (2010) 2330–2340. https://doi.org/10.1002/ijc.24921.
[10] L.J. Bendall, K.F. Bradstock, G-CSF: From granulopoietic stimulant to bone marrow stem cell mobilizing agent, Cytokine Growth Factor Rev. 25 (2014) 355–367. https://doi.org/10.1016/j.cytogfr.2014.07.011.
[11] T.M. Savarese, K. Mitchell, C. McQuain, C.L. Campbell, R. Guardiani, J. Wuu, C. Ollari, F. Reale, B.E. Nelson, A. Chen, P.J. Quesenberry, Coexpression of granulocyte colony stimulating factor and its receptor in primary ovarian carcinomas, Canc. Lett. 162 (2001) 105–115. https://doi.org/10.1016/S0304-3835(00)00623-6.
[12] A. Chakraborty, S. Guha, Granulocyte colony-stimulating factor/granulocyte colony-stimulating factor receptor biological axis promotes survival and growth of bladder cancer cells, Urology 69 (2007) 1210–1215. https://doi.org/10.1016/j.urology.2007.02.035.
[13] J. Morales-Arias, P.A. Meyers, M.F. Bolontrade, N. Rodriguez, Z. Zhou, K. Reddy, A.J. Chou, N.V. Koshkina, E.S. Kleinerman, Expression of granulocyte-colony-stimulating factor and its receptor in human Ewing sarcoma cells and patient tumor specimens: potential consequences of granulocyte-colony-stimulating factor administration, Cancer 110 (2007) 1568–1577. https://doi.org/10.1002/cncr.22964.
[14] B. Yeo, A.D. Redfern, K.A. Mouchemore, J.A. Hamilton, R.L. Anderson, The dark side of granulocyte-colony stimulating factor: a supportive therapy with potential to promote tumour progression, Clin. Exp. Metastasis 35 (2018) 255–267. https://doi.org/10.1007/s10585-018-9917-7.
[15] M. Hollmén, S. Karaman, S. Schwager, A. Lisibach, A.J. Christiansen, M. Maksimow, Z. Varga, S. Jalkanen, M. Detmar, G-CSF regulates macrophage phenotype and associates with poor overall survival in human triple-negative breast cancer, OncoImmunology 5 (2015), e1115177. https://doi.org/10.1080/2162402X.2015.1115177.
[16] M. Kowanetz, X. Wu, J. Lee, M. Tan, T. Hagenbeek, X. Qu, L. Yu, J. Ross, N. Korsisaari, T. Cao, H. Bou-Reslan, D. Kallop, R. Weimer, M.J. Ludlam, J.S. Kaminker, Z. Modrusan, N. van Bruggen, F.V. Peale, R. Carano, Y.G. Meng, N. Ferrara, Granulocyte-colony stimulating factor promotes lung metastasis through mobilization of Ly6G+Ly6C+ granulocytes, Proc. Natl. Acad. Sci. U.S.A. 107 (2010) 21248–21255. https://doi.org/10.1073/pnas.1015855107.
[17] S.B. Coffelt, K. Kersten, C.W. Doornebal, J. Weiden, K. Vrijland, C.S. Hau, N.J.M. Verstegen, M. Ciampricotti, L.J.A.C. Hawinkels, J. Jonkers, K.E. de Visser, IL-17-producing γδ T cells and neutrophils conspire to promote breast cancer metastasis, Nature 522 (2015) 345–348. https://doi.org/10.1038/nature14282.
[18] C.J. Aslakson, F.R. Miller, Selective events in the metastatic process defined by analysis of the sequential dissemination of subpopulations of a mouse mammary tumor, Canc. Res. 52 (1992) 1399–1405.
[19] P.D. Hsu, E.S. Lander, F. Zhang, Development and applications of CRISPR-Cas9 for genome engineering, Cell 157 (2014) 1262–1278. https://doi.org/10.1016/j.cell.2014.05.010.
[20] M.A. Moreno-Mateos, C.E. Vejnar, J.D. Beaudoin, J.P. Fernandez, E.K. Mis, M.K. Khokha, A.J. Giraldez, CRISPRscan: designing highly efficient sgRNAs for CRISPR-Cas9 targeting in vivo, Nat. Methods 12 (2015) 982–988. https://doi.org/10.1038/nmeth.3543.
[21] J. Gao, B.A. Aksoy, U. Dogrusoz, G. Dresdner, B. Gross, S.O. Sumer, Y. Sun, A. Jacobsen, R. Sinha, E. Larsson, E. Cerami, C. Sander, N. Schultz, Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal, Sci. Signal. 6 (2013) pl1, https://doi.org/10.1126/scisignal.2004088.
[22] E. Cerami, J. Gao, U. Dogrusoz, B.E. Gross, S.O. Sumer, B.A. Aksoy, A. Jacobsen, C.J. Byrne, M.L. Heuer, E. Larsson, Y. Antipin, B. Reva, A.P. Goldberg, C. Sander, N. Schultz, The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data, Canc. Discov. 2 (2012) 401–404. https://doi.org/10.1158/2159-8290.CD-12-0095.
[23] A.C. Leal, D.M. Mizurini, T. Gomes, N.C. Rochael, E.M. Saraiva, M.S. Dias, C.C. Werneck, M.S. Sielski, C.P. Vicente, R.Q. Monteiro, Tumor-derived exosomes induce the formation of neutrophil extracellular traps: implications for the establishment of cancer-associated thrombosis, Sci. Rep. 7 (2017) 6438. https://doi.org/10.1038/s41598-017-06893-7.
[24] J.D. Waight, Q. Hu, A. Miller, S. Liu, S.I. Abrams, Tumor-derived G-CSF facilitates neoplastic growth through a granulocytic myeloid-derived suppressor cell-dependent mechanism, PLoS One 6 (2011), e27690. https://doi.org/10.1371/journal.pone.0027690.
[25] T. Welte, I.S. Kim, L. Tian, X. Gao, H. Wang, J. Li, X.B. Holdman, J.I. Herschkowitz, A. Pond, G. Xie, S. Kurley, T. Nguyen, L. Liao, L.E. Dobrolecki, L. Pang, Q. Mo, D.P. Edwards, S. Huang, L. Xin, J. Xu, Y. Li, M.T. Lewis, T. Wang, T.F. Westbrook, J.M. Rosen, X.H. Zhang, Oncogenic mTOR signalling recruits myeloid-derived suppressor cells to promote tumour initiation, Nat. Cell Biol. 18 (2016) 632–644. https://doi.org/10.1038/ncb3355.
[26] R. Ramachandran, K. Mihara, H. Chung, B. Renaux, C.S. Lau, D.A. Muruve, K.A. DeFea, M. Bouvier, M.D. Hollenberg, Neutrophil elastase acts as a biased agonist for proteinase-activated receptor-2 (PAR2), J. Biol. Chem. 286 (2011) 24638–24648. https://doi.org/10.1074/jbc.M110.201988.
[27] L. Ge, S.K. Shenoy, R.J. Lefkowitz, K. DeFea, Constitutive protease-activated receptor-2-mediated migration of MDA MB-231 breast cancer cells requires both beta-arrestin-1 and -2, J. Biol. Chem. 279 (2004) 55419–55424. https://doi.org/10.1074/jbc.M410312200.
[28] R.J. Lohman, A.J. Cotterell, G.D. Barry, L. Liu, J.Y. Suen, D.A. Vesey, D.P. Fairlie, An antagonist of human protease activated receptor-2 attenuates PAR2 signaling, macrophage activation, mast cell degranulation, and collagen-induced arthritis in rats, Faseb. J. 26 (2012) 2877–2887. https://doi.org/10.

1096/fj.11-201004.
[29] S. Boitano, J. Hoffman, A.N. Flynn, M.N. Asiedu, D.V. Tillu, Z. Zhang, C.L. Sherwood, C.M. Rivas, K.A. DeFea, J. Vagner, T.J. Price, The novel PAR2 ligand C391 blocks multiple PAR2 signalling pathways in vitro and in vivo, Br. J. Pharmacol. 172 (2015) 4535–4545. https://doi.org/10.1111/bph.13238.
[30] T.C. Carneiro-Lobo, F. Schaffner, J. Disse, H. Ostergaard, I.M. Francischetti, R.Q. Monteiro, W. Ruf, The tick-derived inhibitor Ixolaris prevents tissue factor signaling on tumor cells, J. Thromb. Haemostasis 10 (2012) 1849–1858. https://doi.org/10.1111/j.1538-7836.2012.04864.x.
[31] A. Chanakira, P.R. Westmark, I.M. Ong, J.P. Sheehan, Tissue factor-factor VIIa complex triggers protease activated receptor 2-dependent growth factor release and migration in ovarian cancer, Gynecol. Oncol. 145 (2017) 167–175. https://doi.org/10.1016/j.ygyno.2017.01.022.
[32] Y. Jiang, M.K. Yau, J. Lim, K.C. Wu, W. Xu, J.Y. Suen, D.P. Fairlie, A potent antagonist of protease-activated receptor 2 that inhibits multiple signaling functions in human cancer cells, J. Pharmacol. Exp. Therapeut. 364 (2018) 246–257. https://doi.org/10.1124/jpet.117.245027.

Update

BBA - Molecular Basis of Disease

Volume 1865, Issue 1, January 2019, Page 262

DOI: https://doi.org/10.1016/j.bbadis.2018.09.017

BBA - Molecular Basis of Disease 1865 (2019) 262

Contents lists available at ScienceDirect

BBA - Molecular Basis of Disease

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

Corrigendum

Corrigendum to "Vascular pentraxin 3 controls arterial thrombosis by targeting collagen and fibrinogen induced platelets aggregation" [Biochim. Biophys. Acta, 1862 (2016) 1182–1190]

F. Bonacina[a], S.S. Barbieri[b], L. Cutuli[a], P. Amadi[b], A. Don[c], M. Sironi[c], S. Tartari[c], A. Mantovani[c], B. Bottazzi[c], C. Garlanda[c], E. Tremoli[a,b], A.L. Catapano[d,*], G.D. Norata[a,e,f,**]

[a] Department of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano, Milan, Italy
[b] IRCCS, Centro Cardiologico Monzino, Milan, Italy
[c] IRCCS, Humanitas Research Foundation, Bruzzano, Milan, Italy
[d] IRCCS Multimedica, Milan, Italy
[e] SISA Centre for the Study of Atherosclerosis, Bassini Hospital, Cinisello B, Milan, Italy
[f] William Harvey Research Institute, Barts and The London School of Medicine & Dentistry, Queen Mary University, London, UK

The authors regret that a wrong grant was acknowledged. The sentence "The financial supports of the European Research Council (ERC project HIIS)" should be changed as follow: "The financial supports of the European Research Council (ERC project PHII, contract N° 669415).

The authors would like to apologise for any inconvenience caused.

DOI of original article: https://doi.org/10.1016/j.bbrc.2018.08.169
* Corresponding author.
** Correspondence to: G.D. Norata, Department of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano, Milan, Italy.
E-mail addresses: alberico.catapano@unimi.it (A.L. Catapano), danilo.norata@unimi.it (G.D. Norata).

https://doi.org/10.1016/j.bbadis.2018.09.017

Available online 16 October 2018