Autoimmunity Reviews 16 (2017) 398–406

Contents lists available at ScienceDirect

Autoimmunity Reviews

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

Review

Molecular analysis of vascular smooth muscle cells from patients with giant cell arteritis: Targeting endothelin-1 receptor to control proliferation☆

Alexis Régent [a,b,1], Kim Heang Ly [c,d,1], Matthieu Groh [a,b], Chabha Khifer [a], Sébastien Lofek [a], Guilhem Clary [a], Philippe Chafey [a], Véronique Baud [e], Cédric Broussard [a], Christian Federici [a], François Labrousse [f], Laura Mesturoux [f], Claire Le Jeunne [b], Elisabeth Vidal [c,d], Antoine Brezin [g], Véronique Witko-Sarsat [a], Loïc Guillevin [b], Luc Mouthon [a,b,*]

[a] Institut Cochin, INSERM U1016, CNRS UMR 8104, LabEx INFLAMEX, Université Paris Descartes, Sorbonne Paris Cité, Paris, France
[b] Pôle de Médecine Interne, Centre de Référence pour les vascularites nécrosantes et la sclérodermie systémique, Hôpital Cochin, Assistance Publique-Hôpitaux de Paris (AP-HP), Paris, France
[c] Service de Médecine Interne A, CHU Dupuytren, Limoges, France
[d] Laboratoire d'Immunologie, EA3842, Faculté de médecine, Limoges, France
[e] Laboratoire NF-κB, Differentiation and Cancer, Université Paris Descartes, Sorbonne Paris Cité, Paris, France
[f] Laboratoire d'Anatomie Pathologie, CHU Limoges, Limoges, France
[g] Service d'Ophtalmologie, Hôpital Cochin, AP-HP, Paris, France

ARTICLE INFO

Article history:
Received 8 December 2016
Accepted 16 December 2016
Available online 14 February 2017

Keywords:
Giant cell arteritis
Vascular smooth muscle cells
Proliferation
Paxillin
Endothelin-1

ABSTRACT

Objective: The pathophysiology of giant cell arteritis (GCA) and the mechanisms underlying vascular remodeling, are poorly understood. We aimed to compare vascular smooth muscle cells (VSMCs) from patients with GCA and controls by a proteomic and gene expression profile approach and to identify the signaling pathways involved in proliferation.

Methods: VSMCs were cultured from temporal artery biopsies (TABs) from patients with biopsy-proven GCA (TAB^+-GCA), biopsy-negative GCA (TAB^--GCA), and diagnosis other than GCA (GCA-control). VSMCs from normal human aorta (HAoSMC) were used as controls. 2D-differential in-gel electrophoresis and Affymetrix chips were used to compare proteomes and gene expression profiles of VSMCs. Proliferation was assessed by BrdU incorporation assay. TAB^+-GCA and GCA-control TABs underwent immunohistochemistry staining for endothelin-1 (ET-1) and receptors ET_AR and ET_BR.

Results: We identified 16, 30 and 2 protein spots differentially expressed between TAB^+-GCA and GCA-control VSMCs, TAB^+-GCA and TAB^--GCA VSMCs and TAB^--GCA and GCA-control VSMCs, respectively (fold change ≥1.5 and $p \leq 0.05$). Among the 153 proteins differentially expressed between TAB^+-GCA and HAoSMC VSMCs, many were linked with ET-1. Genes differentially expressed between TAB^+-GCA and GCA-control VSMCs were involved in proliferation. ET-1 was identified as a link between genes of interest. Proliferation was reduced for TAB^+-GCA VSMCs on treatment with the endothelin antagonist macitentan and its active metabolite. Patients showing transmural expression of ET-1 in temporal artery lesions received a significantly higher glucocorticoid daily dose after 6-month follow-up.

Conclusion: Inhibiting the proliferation with macitentan, combined with glucocorticoids, might be a promising therapeutic approach for patients with GCA.

Abbreviations: α-SMA, α-smooth muscle actin; 2D-DIGE, 2D differential in-gel electrophoresis; ET-1, endothelin 1; GCA, giant cell arteritis; HAoSMC, vascular smooth muscle cell from normal human aorta; MS, mass spectrometry; PCA, principal component analysis; PDGF, platelet-derived growth factor; PPIA, peptidylpropyl isomerase A; TA, temporal artery; TAB, temporal artery biopsy; VSMC, vascular smooth muscle cells.

☆ **Disclosure**: LM received grants, is investigator and consultant for Actelion Pharmaceuticals

* Corresponding author at: INSERM U1016, CNRS UMR 8104, 8 rue Mechain, 75014 Paris, France.
E-mail addresses: alexisregent@hotmail.com (A. Régent), kim.ly@chu-limoges.fr (K.H. Ly), matthieu.groh@aphp.fr (M. Groh), Ckiefer@yahoo.fr (C. Khifer), sebastien.lofek@inserm.fr (S. Lofek), guilhem.clary@inserm.fr (G. Clary), philippe.chafey@inserm.fr (P. Chafey), veronique.baud@inserm.fr (V. Baud), cedric.broussard@inserm.fr (C. Broussard), christian.federici@inserm.fr (C. Federici), francois.labrousse@unilim.fr (F. Labrousse), laura.mesturoux@chu-limoges.fr (L. Mesturoux), claire.le-jeunne@aphp.fr (C. Le Jeunne), elisabeth.vidal@unilim.fr (E. Vidal), antoine.brezin@aphp.fr (A. Brezin), veronique.witko@inserm.fr (V. Witko-Sarsat), loic.guillevin@aphp.fr (L. Guillevin), luc.mouthon@aphp.fr (L. Mouthon).

[1] Both authors contributed equally to the work.

http://dx.doi.org/10.1016/j.autrev.2017.02.006
1568-9972/

Contents

1. Introduction

Giant cell arteritis (GCA) is a primary vasculitis that occurs in patients older than 50 [1]. It specifically involves the aorta [2] and external carotid arteries and their branches, with intimal hyperplasia and luminal obstruction leading to ischemic manifestations (e.g. temporal headaches, jaw claudication, scalp tenderness). The initial prognosis is visual impairment with blurry vision as well as transient or permanent sight loss in as much as 20% of patients [3,4]. Early diagnosis is required for initiating glucocorticoid treatment, even though it has a limited efficacy on vision if already impaired [5].

Pathological features of temporal artery biopsies (TAB) consist of mononuclear T helper 1 (Th-1) and Th-17 cell infiltrates of the adventitia, internal elastic lamina disruption with macrophages and multinucleated cells and intimal hyperplasia [6]. Macrophages produce platelet-derived growth factor (PDGF) and vascular endothelial growth factor (thus leading to vessel wall thickening) [7,8], and high levels of reactive oxygen species that target vascular smooth muscle cells (VSMCs) [9]. The imbalance between levels of matrix metalloproteases (MMPs) and the tissue Inhibitors of MMPs [10] contributes to the remodeling of the vessel wall [11]. Endothelin 1 (ET-1), a powerful vasoconstrictor, and the endothelin receptors ET_AR and ET_BR, were found expressed in vascular lesions from GCA patients [12,13] and high plasma levels of ET-1 were associated with risk of visual impairment [12,14].

We recently described that VSMC protein content and phenotypic differentiation varies along the arterial tree and during pathological process [15]. Thus, we performed a proteomic analysis and gene expression profile of VSMCs isolated from temporal artery biopsies (TABs) from patients with GCA and controls. We also investigated the implication of the endothelin receptors ET_AR and ET_BR in the proliferation of VSMCs.

2. Patients and methods

2.1. Patients

Between January 2010 and June 2012, we included 122 consecutive patients with suspected GCA at the time of TAB in two different centers (Limoges and Paris) (collection dc-2010-1079). VSMCs from patients with GCA were isolated from sterile, freshly obtained surgical TABs from patients with suspected GCA. We then selected 12 consecutive patients with successful VSMCs culture: 4 with TAB-proven GCA (TAB^{+}-GCA), 4 fulfilling the American College of Rheumatology criteria for GCA but without histological evidence of vasculitis (TAB^{-}-GCA), and 4 with alternate diagnoses (GCA-control). Clinical and biological data detailed in Additional Table 1 were recorded at the time of TAB and updated with the TAB results and final diagnosis. All patients and controls gave their written informed consent to participate. The ethics committee of Cochin hospital approved the study.

2.2. VSMCs

Briefly, temporal arteries were dissected and adventitia and media were mechanically separated. Media was digested by collagenase and elastase for 90 min before seeding in cell culture flasks (Falcon, Becton Dickinson, Franklin Lakes, NJ, USA). SMC origin was assessed by positive α-SMA staining and negative CD90 staining in indirect immunofluorescence experiments. VSMCs from normal human aortas (HAoSMC) from 4 different healthy Caucasian donors older than 50 years were used as controls (Promocell GmbH, Heidelberg, Germany).

HAoSMCs and VSMCs cultured in SMC growth medium 2 (Promocell GmbH) to passage 4 were harvested and proteins were extracted as described [16].

2.3. 2D differential in-gel electrophoresis (2D-DIGE) and protein identification by mass spectrometry (MS)

VSMC samples were labelled with fluorescent dyes and separated by 2D electrophoresis. Gels were then analysed and protein spots with differential expression among VSMC subtypes were excised, in-gel trypsin-digested and identified by MS. 2D-DIGE, in-gel trypsin digestion, and MS protein identification were adapted from [16] and are detailed in Additional file 1.

2.4. RNA extraction in culture cells and chip analysis

For chip experiments, RNA was extracted from cells at passage 4 by the Trizol method [17] as detailed in Additional file 1. After quality control, pan-genomic Affymetrix genechips (Affymetrix, Santa Clara, CA, USA) were used according to the manufacturer's instructions. For RNA analysis, data were normalized by using the Robust Multi-array Average (RMA) algorithm in Bioconductor with the ENSG custom CDF vs 15 [18].