Human Atheromatous Plaques Expressed Sensing Adaptor STING, a Potential Role in Vascular Inflammation Pathogenesis

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Introduction

Atherogenesis is a complex physiological process that involves inflammation, and it is a significant contributor to plaque development and plaque vulnerability. Immune activation can be behind the increased inflammation in atherosclerosis.[1] In many organisms, detecting foreign DNA is an essential part of immunity. The cyclic GMP–AMP synthase (cGAS)–stimulator of interferon genes (STING) pathway has emerged as an effective mechanism for connecting DNA sensing to the production of potent innate immune defense programs and it plays a substantial role in this process in mammalian cells.[2] The allosteric activation of cGAS' catalytic activity by binding to double-stranded DNA leads to the formation of 2′,3′-cyclic GMP–AMP (cGAMP), a second messenger molecule and potent agonist of STING. The activation of the cGAS–STING pathway is triggered by a fundamental constituent of life (particularly DNA), and it lacks any pathogen-specific characteristics, which distinguishes it from several other innate immune signaling mechanisms. As a result, cGAS identifies a wide range of DNA species, both foreign and self-derived.

Even though pathological process inducers are disease-specific, there is evidence for the cGAS–STING pathway's role as a driver of both (hyper-)acute and chronic, low-grade inflammatory states associated with a variety of illnesses.[3] This role is not unexpected due to its fundamental function as a ubiquitous and sensitive mechanism for out-of-context DNA recognition. Furthermore, preliminary evidence suggested that intracellular trafficking pathway abnormalities can significantly impact STING activity, and it contributes to inappropriate immune activation. Although chronic low-grade inflammatory stages have been associated with atherosclerosis, the cGAS–STING pathway has not been explored in this relevant pathophysiological process. Here, we explored the expression of STING protein in the atheriosclerotic (ATC) plaque of human patients who underwent carotid endarterectomy.

Methods

To describe whether STING protein is expressed in ATC plaques and to characterize which ATC plaques' cells expressed STING protein, immunostaining against STING was performed on ATC plaques from 19 patients who underwent endarterectomy. The Ethical Committee of Hospital Universitari Arnau de Vilanova (Lleida, Spain; Approval number: CEIC-1282) approved the study. According to standard procedures, ATC plaques were paraffin-embedded, and

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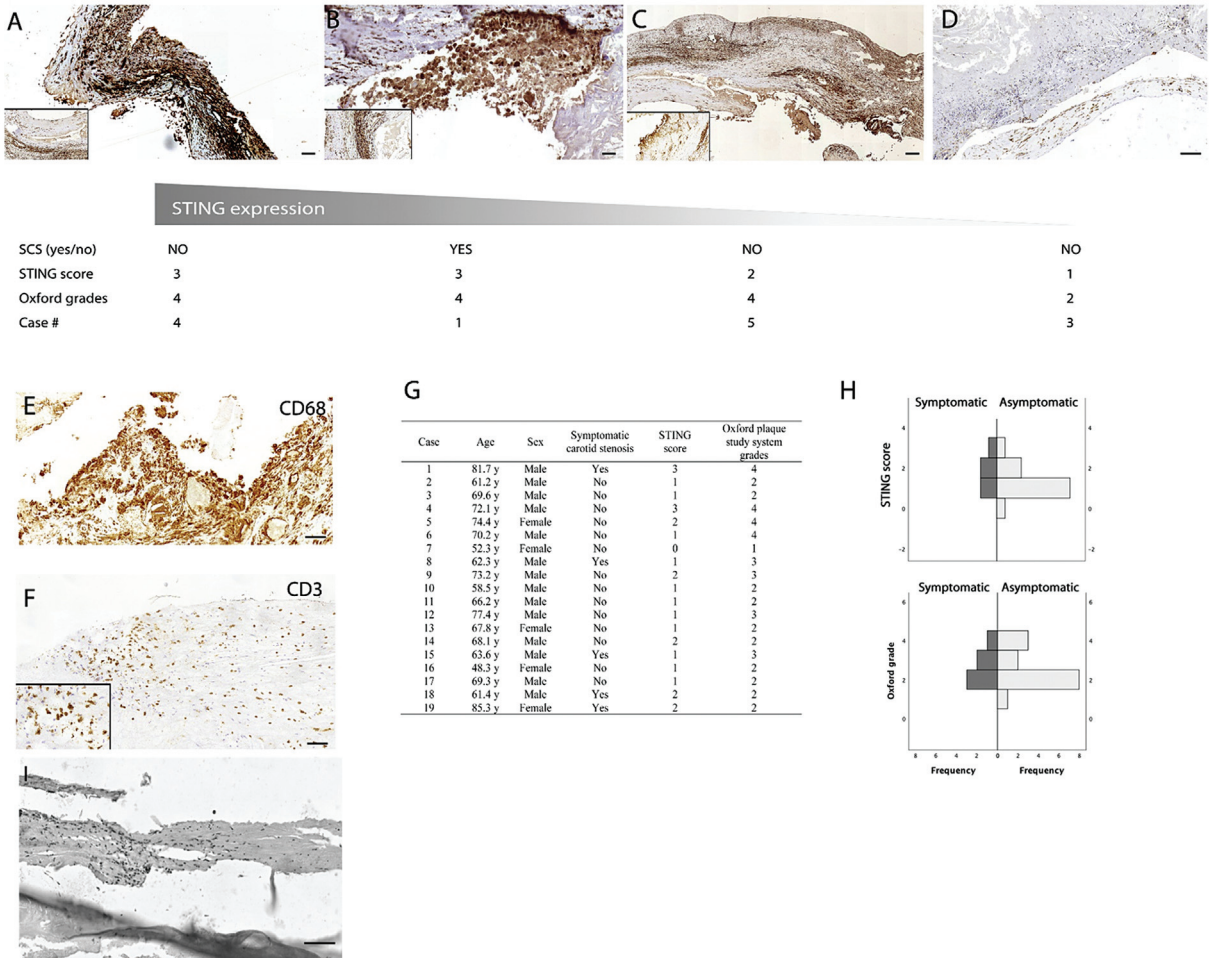
human spleen paraffin-embedded samples were used as positive controls (data not shown). Negative controls consisting of the omission of the primary antibody resulted in the disappearance of the signal. Primary rabbit anti-human STING antibody (1:150 dilution, #19851–1-AP, Proteintech) was used and the reaction was visualized by EnVision FLEX Detection kit (Agilent Technologies). T-cells and macrophages were visualized by immunostaining using primary polyclonal anti-human CD3 (Agilent DAKO) and polyclonal anti-human CD68 (clone PG-M1, Agilent DAKO), respectively. Samples were counterstained with hematoxylin. Images were obtained using a DMI1000 light microscope (Leica). STING protein expression was variable and heterogeneous among patients, for that samples were scored using a semi-quantitative immunoreactivity scoring system, which was defined as: 0: no signal; 1: weak signal; 2: moderate signal; 3: strong signal. The slides were scored by an experienced senior pathologist (J.P.).

Results and Discussion

STING expression was detected in the ATC plaques (►**Fig. 1A**–**D**). The atheromatous core showed numerous foam cells, lymphocytes close to the lipid core in all checked carotid arteries. In serial sections of those areas, previous immunohistochemical analysis with CD68 and CD3 antibodies localized the macrophages and T-cells of the plaques (►**Fig. 1E**, **F**). Regarding cellular distribution, STING staining was mainly localized in the cell cytoplasm of different immune cells. Most of the STING expression was noted in macrophages and T-cells. Of note, endothelial cells were constantly positive in all cases analyzed. STING expression was stronger in the complicated ATC plaques near the cell debris and hemorrhagic foci. Immunostaining samples with antiactivated caspase-3 reveal no relationship with STING expression (data not shown). Negative controls (►**Fig. 1I**) reinforce the specificity of the findings.



Case	Age	Sex	Symptomatic carotid stenosis	STING score	Oxford plaque study system grades
1	81.7 y	Male	Yes	3	4
2	61.2 y	Male	No	1	2
3	69.6 y	Male	No	1	2
4	72.1 y	Male	No	3	4
5	74.4 y	Female	No	2	4
6	70.2 y	Male	No	1	4
7	52.3 y	Female	No	0	1
8	62.3 y	Male	Yes	1	3
9	73.2 y	Male	No	2	3
10	58.5 y	Male	No	1	2
11	66.2 y	Male	No	1	2
12	77.4 y	Male	No	1	3
13	67.8 y	Female	No	1	2
14	68.1 y	Male	No	2	2
15	63.6 y	Male	Yes	1	3
16	48.3 y	Female	No	1	2
17	69.3 y	Male	No	1	2
18	61.4 y	Male	Yes	2	2
19	85.3 y	Female	Yes	2	2

Fig. 1 STING expression in atheromatous plaques. Immunostaining with anti-STING antibody in four ATC plaques showed a gradient of expression, strong STING expression (**A, B**), moderate STING expression (**C**), and weak STING expression (**D**). Immunostaining of successive sections with CD68 antibody showed abundant macrophages in the atherosclerotic plaque (**E**). Immunostaining against CD3 antibody showed T cell expression in the atherosclerotic plaque (**F**). STING score: 0 no signal, 1 weak signal, 2 moderate, and 3 intense staining. Characteristics of all evaluated cases ($n = 19$): age, sex, symptomatic carotid stenosis (yes/no), STING score (0: no signal; 1: weak signal; 2: moderate signal; 3: strong signal), and Oxford plaque study system grades (grade 1: stable plaque; grade 2: ATC plaque predominantly stable; grade 3: unstable plaques with intact; grade 4: unstable plaques with rupture cap) (**G**).Frequency plots grouped by symptomatic and asymptomatic showed a relationship between STING score and Oxford grade, mainly in asymptomatic patients (**H**). Negative control for anti-STING staining. Sections were counterstained with hematoxylin (**I**). Scale bar: (A) 100 µm, (B, E, F) 50 µm, (C, D,I) 200 µm.

These results showed the occurrence of cytosolic STING expression in human atheromatous plaques. Regarding the exact driver of STING expression as a cytosolic DNA-sensing adaptor, one may account for the existence of a proinflammation, senescence-prone cellular milieu. Also, single-stranded DNA—in close relationship with mitochondrial and nuclear DNA damage (evidenced by an increase of γH2AX and p53)—could be activating STING signaling (as shown in the murine atherosclerosis ApoE −/− model, mainly in macrophages).[4] Cytosolic DNA may also arise from endocytosis of dying cells, plaque-present pathogens or other damage-associated molecular patterns, including those derived from cell senescence. Given the increased interest in senescent cells in different disease contexts, including atherosclerosis,[5] more research is needed into the relationship between inflammatory senescence and the cGAS–STING pathway. Excessive inflammation, failure inflammation resolution, and premature senescence are significant contributors to plaque development and vulnerability. Also, the reduction in mitochondrial function is a hallmark of the aging process and is age-dependent. Mitochondrial defects may also play a role in atherosclerosis-associated STING upregulation.[6] Therefore it is tempting to believe that abnormal cGAS–STING signaling plays a role in atherogenesis. However, the underlying mechanisms are not well known yet. Indeed, as a limitation of our work, we should acknowledge that the number of samples is too limited to draw any conclusions regarding the correlation of STING expression with the disease stage. Last but not least, this cGAS–STING pathway is targeted by several drugs, and its potential exploration would open novel venues to develop therapeutic targets to treat atherosclerosis.

Author Contributions

F.P., N.T., and M.P-.O. conceived the study. G.A. and J.P. designed the experiments. N.T., J.P., G.A.: sample processing, data analysis, and data interpretation. G.A., F.P., and M. P-.O.: drafted the manuscript. M.P.O. and F.P. procured funding. All authors critically revised and approved the final version of the manuscript.

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Conflict of Interest

None declared.

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