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Human monocytes and macrophages express NADPH oxidase 5; a potential source of reactive oxygen species in atherosclerosis

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ABSTRACT

Monocytes (Mon) and Mon-derived macrophages (Mac) orchestrate important oxidative and inflammatory reactions in atherosclerosis by secreting reactive oxygen species (ROS) due, in large part, to the upregulated NADPH oxidases (Nox). The Nox enzymes have been extensively investigated in human Mon and Mac. However, the expression and functional significance of the Nox5 subtypes is not known. We aimed at elucidating whether Nox5 is expressed in human Mon and Mac, and examine its potential role in atherosclerosis. Human monocytic THP-1 cell line and $CD14^+$ Mon were employed to search for Nox5 expression. RT-PCR, Western blot, lucigenin-enhanced chemiluminescence and dihydroethidium assays were utilized to examine Nox5 in these cells. We found that Nox5 transcription variants and proteins are constitutively expressed in THP-1 cells and primary $CD14^+$ Mon. Silencing of Nox5 protein expression by siRNA reduced the Ca^{2+}-dependent Nox activity and the formation of ROS in Mac induced by A23187, a selective Ca^{2+} ionophore. Exposure of Mac to increasing concentrations of IFNγ (5–100 ng/ml) or oxidized LDL (5–100 μg/ml) resulted in a dose-dependent increase in Nox5 protein expression and elevation in intracellular Ca^{2+} concentration. Immunohistochemical staining revealed that Nox5 is present in $CD68^+$ Mac-rich area within human carotid artery atherosclerotic plaques. To the best of our knowledge, this is the first evidence that Nox5 is constitutively expressed in human Mon. Induction of Nox5 expression in IFNγ- and oxidized LDL-exposed Mac and the presence of Nox5 in Mac-rich atheroma are indicative of the implication of Nox5 in atherogenesis.

1. Introduction

The involvement of monocytes (Mon) and Mon-derived macrophages (Mac) in atherosclerosis is a topic of continual interest because they orchestrate important inflammatory and oxidative reactions by secreting pro-inflammatory cytokines, chemokines and detrimental reactive oxygen species (ROS) [1].

Mac are important sources of ROS, mainly generated by activated NADPH oxidases (Nox). Nox-derived ROS play an important role in regulating signal transduction pathways but their overproduction is acutely harmful to cells (particularly in cardiovascular diseases) by mechanisms that are not fully understood [2].

Nox represent a family of hetero-oligomeric enzymes comprising seven members (Nox1, Nox2, Nox3, Nox4, Nox5, Duox1, and Duox2), whose unique function is the production of ROS [3]. Besides Nox2, the expression of Nox1 and Nox4 has been reported in Mon and Mac [4,5]. It is yet to be revealed whether Nox5 exists and is functional in Mon and Mac.

The human gene encoding Nox5 is located on chromosome 15q23. Alternative promoter usage leads to the generation of two structurally related Nox5 enzymes containing the Nox2-type catalytic core protein and a variable NH_2 terminus domain, namely Nox5-L (Long) and Nox5-S (Short). The Nox5-L contains an NH_2-terminal calmodulin-like domain comprising four Ca^{2+}-binding EF-hands structures responsible for enzyme activation, whereas Nox5-S (Nox5ε), lacks this domain. Four splice variants of Nox5-L, namely Nox5α, Nox5β, Nox5γ, and Nox5δ have been identified in humans [6].

Nox5 function is regulated by the intracellular Ca^{2+} mobilization and influx [15,16], and phosphorylation [7–11]. The extent of Nox5-dependent ROS production is closely related to the level of its

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protein expression [12]. Multiple pro-inflammatory transcriptional mechanisms have been described to directly or indirectly affect Nox5 expression [13,14].

In this study, we aimed at elucidating whether Nox5 and its related splice variants are expressed in human Mon, and to examine their potential implication in atherosclerosis. We provide evidence that Nox5 is constitutively expressed in human Mon, Nox5 contributes to ROS formation in Mac, interferon γ (IFNγ) and oxidized low-density lipoprotein (oxLDL) induce its protein expression, and is localized in Mac-rich area within the atherosclerotic plaques in human carotid arteries. To the best of our knowledge, this is the first study that demonstrates the existence of Nox5 in Mon and Mac suggesting its potential implication in atherogenesis.

2. Materials and methods

2.1. Materials

Standard chemicals and reagents were purchased from Sigma–Aldrich if not stated otherwise. Antibodies and siRNA were obtained from Santa Cruz Biotechnology. Dr. Anca Sima kindly provided oxLDL.

2.2. Cell culture and tissue samples

Human THP-1 monocytic cell line was obtained from American Type Culture Collection (ATCC). Human peripheral blood mononuclear cells (PBMC) were freshly isolated from healthy donors (Blood Transfusion Center Bucharest) as previously described [15]. The $CD14^+$ Mon population was isolated from PBMC (Miltenyi Biotech). Mon differentiation to Mac was induced by exposing the cells (3 days) to 100 nM phorbol-12-myristate-13-acetate (PMA). Human aortic smooth muscle cells (SMCs) [16] were used as positive control for Nox5 expression. RAW264.7 murine Mac (ATCC) and mouse (C57BL/6J) aortic cryosections were employed as negative controls. The animal experiments were conducted in accordance with the EU Directive 63/2010. Human atherosclerotic plaques were obtained as discarded tissue from patients undergoing carotid endarterectomy (University Hospital Bucharest). The study was performed in agreement with the ethical directives for medical research involving human subjects (World Medical Association Declaration of Helsinki). Written informed consent was obtained from all patients. The ethical committee at the ICBP "Nicolae Simionescu" approved the study protocol.

2.3. Reverse-transcription-polymerase chain reaction (RT-PCR)

The RT-PCR assays were done by using standard kits (Invitrogen). The PCR conditions were: denaturation at 95 °C, annealing at 65 °C, and extension at 72° C, 45 s each, for 40 cycles. The primer sequences were: Nox5 (332 bp) sense: 5′-AGAGTCAAAGGTCGTCCAAGGG-3′, antisense: 5′- AGCAGGCTCACAAACCACTCG-3′; GAPDH (450 bp) sense: 5′-ACCACAGTCCATGCCATCAC-3′, antisense: 5′-TCCACCACCCTGTTGCTGTA-3′. The primers used to detect the Nox5α (326 bp), Nox5β (411 bp), Nox5δ (661 bp), and Nox5γ (475 bp) variants were as in Ref. [17].

2.4. Western blot analysis

Western blot assays were done as in Ref. [18] using Nox1 (sc-25545), Nox2 (sc-5827), Nox4 (sc-30141), Nox5 (sc-67006) and β-Actin (sc-47778) primary antibodies. The protein bands were detected with a digital image acquisition system (ImageQuant LAS 4000, Fujifilm). The quantification of Nox proteins was done by normalization to β-Actin protein using the TotalLab™ software and expressed as arbitrary units.

2.5. Measurement of ROS formation

The NADPH-dependent $O_2^{\bullet-}$ production was measured in membrane fractions isolated from cultured THP-1 Mac employing the lucigenin-enhanced chemiluminescence assay [19]. The chemiluminescence signal was detected in the absence/presence of 1 mM $CaCl_2$/ethylenediaminetetraacetic acid (EDTA). The Nox activity was calculated from the ratio of mean light units to protein concentration, and expressed as arbitrary units.

The intracellular formation of ROS in THP-1 Mac was assessed by dihydroethidium (DHE) assay [20]. The intracellular ROS production was calculated from the ratio of relative fluorescence units to protein concentration and expressed as arbitrary units.

2.6. Transfection of siRNA

Cultured THP-1 Mac (at ≈50% confluence in 60 mm Ø tissue culture plates) were transfected with 20 nM of control siRNA (C siRNA, sc-37007) or Nox5 siRNA (sc-45486) using Hiperfect transfection reagent (Qiagen). The efficiency of siRNA to induce Nox5 protein down-regulation was monitored by Western blot, 48 h after transfection.

2.7. Evaluation of atherosclerotic lesions

The human carotid atherosclerotic lesions were analyzed after Oil Red O staining as previously described [21]. Carotid cryosections (7 μm) were taw-mounted onto microscope slides and stained with Oil Red O solution. Sections were counter-stained with hematoxylin and images were taken using an inverted phase contrast microscope (Carl Zeiss Observer D1).

2.8. Immunohistochemistry

Serial cryosections were subjected to immunohistochemical (IHC) analysis using rabbit IgG and mouse IgG Vectastain™ ABC kits (Vector Laboratories). The sections were incubated with the primary antibodies against Nox5 (sc-67006), CD68 (sc-9139) or α smooth muscle actin (αSMA, sc-32251), followed by specific biotin-conjugated secondary antibodies and streptavidin-horseradish peroxidase. The specimens were counter-stained with hematoxylin and photographed using an inverted phase contrast microscope (Carl Zeiss Observer D1).

2.9. Determination of intracellular concentration of Ca^{2+}

The Fura 2-AM was used to measure the intracellular calcium concentration $[Ca^{2+}]_i$ in Mac as described in Ref. [22]. $[Ca^{2+}]_i$ was calculated from the ratio of relative fluorescence units to protein concentration and expressed as arbitrary units.

2.10. Statistical analysis

Data derived from a minimum of 3 independent experiments were expressed as means ± standard deviation. Statistical analysis was done by one-way ANOVA and Tukey's range test; $P < 0.05$ was considered statistically significant.

3. Results

3.1. Human monocytes express constitutively Nox5 variants

To determine whether Nox5 is expressed in human Mon, RT-PCR assays were done employing primers common to all Nox5 transcription variants and primer sets [17] designed to amplify the Nox5α, Nox5β, Nox5δ, and Nox5γ mRNA splicing products. A schematic depiction of the amino acid sequence variations within NH_2-terminal region of the Nox5 isoforms is shown in Fig. 1A. The expression of Nox5 was analyzed in both THP-1 monocytic cell line and primary $CD14^+$ Mon. The RT-PCR assays revealed that Nox5 mRNA is present in both THP-1 cells and $CD14^+$ Mon. The specificity of PCR products was confirmed in human aortic SMCs, taken as positive control for Nox5 expression. The mRNA expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as amplification control (Fig. 1B).

To investigate which Nox5 transcription variants are expressed in Mon splice-variant specific primers were employed. The results showed that Mon expressed Nox5α and Nox5β whereas SMCs expressed Nox5α, Nox5β, Nox5δ, and Nox5γ (Fig. 1B).

To examine whether human Mon also express Nox5 proteins, we used a rabbit polyclonal antibody designed to detect all the Nox5 subtypes. The full-length amino acid sequence (NP_078781.3) of Nox5 protein and the immunogen are presented in Fig. 1C. The polyclonal antibody intensely labeled the protein bands of ≈90 kDa in both Mon and SMCs. An additional protein band of ≈65 kDa was detected both in THP-1 and $CD14^+$ Mon. No immunostaining of Nox5 proteins was observed in cell lysates derived from cultured RAW264.7 Mac (Fig. 1D). Based on the predicted molecular weight (Nox5α ≈ 84 kDa, Nox5β ≈ 82 kDa, Nox5δ ≈ 85 kDa, Nox5γ ≈ 86 kDa, Nox5ε ≈ 65 kDa) one can assume that the ≈90 kDa protein band corresponds to Nox5α/β subtypes whereas the ≈65 kDa protein band matches the expected molecular weight of Nox5ε isoform.

3.2. Nox5 contributes to Ca^{2+}-dependent Nox activity and ROS formation in Mac

Transient transfection of THP-1 Mac with Nox5 siRNA was employed to investigate the contribution of Nox5 in mediating ROS formation. The Ca^{2+}-generating ROS production was measured in membrane fractions and intact cells, 72-h after siRNA transfection. $CaCl_2$ induced a significant increase in Nox activity (≈2-fold). Notably, the Ca^{2+}-dependent Nox activity was significantly down-regulated by Nox5 siRNA and by the Ca^{2+} chelator, EDTA (Fig. 2A and B).

To further investigate the implication of Nox5 in mediating Ca^{2+}-induced ROS formation in Mac, the cells were loaded with DHE probe and exposed for an additional 1 h to vehicle (DMSO) or 1 μM A23187 (1 h), a selective Ca^{2+} ionophore. The results showed that silencing of Nox5 led to a significant reduction (≈30%) of A23187-induced DHE fluorescence (Fig. 2C). No significant changes in Ca^{2+}-dependent Nox activity or Ca^{2+}-dependent DHE signal were detected in C siRNA-transfected cells. The decrease in Nox5 proteins upon siRNA transfection in THP-1 Mac was confirmed by

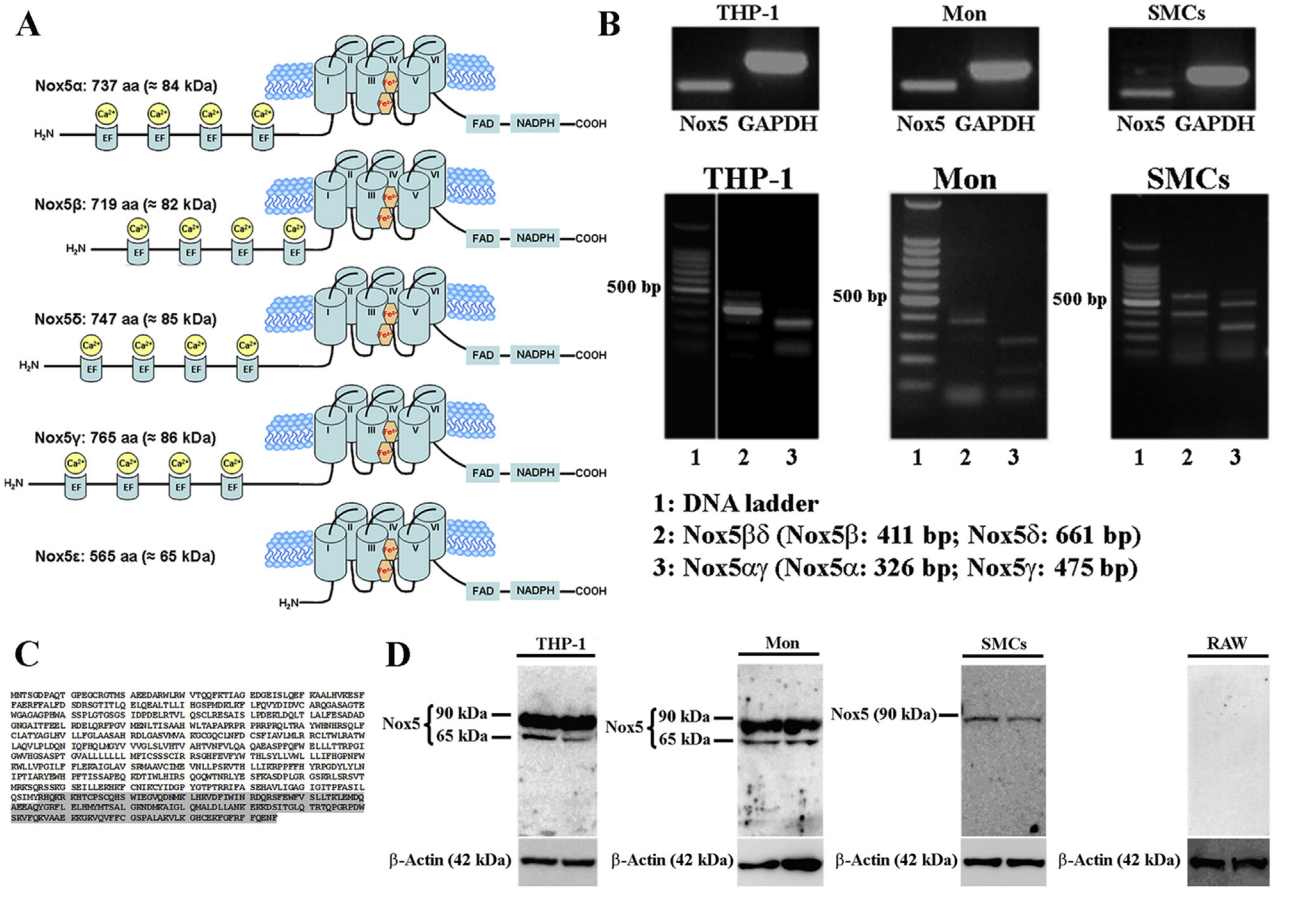

Fig. 1. Human monocytes express Nox5. (A) Schematic depiction of Nox5 subtypes showing the variations within NH_2-terminal region. (B) RT-PCR analysis of the Nox5 mRNA and Nox5 transcription variants in THP-1 cell line (THP-1), primary $CD14^+$ Mon and SMCs (positive control). (C) The Nox5 protein amino acid sequence with immunogen highlighted. (D) Western blot analysis of Nox5 proteins in THP-1, CD14 + Mon, SMCs, and RAW264.7 Mac (negative control). Representative data from 4 independent experiments.

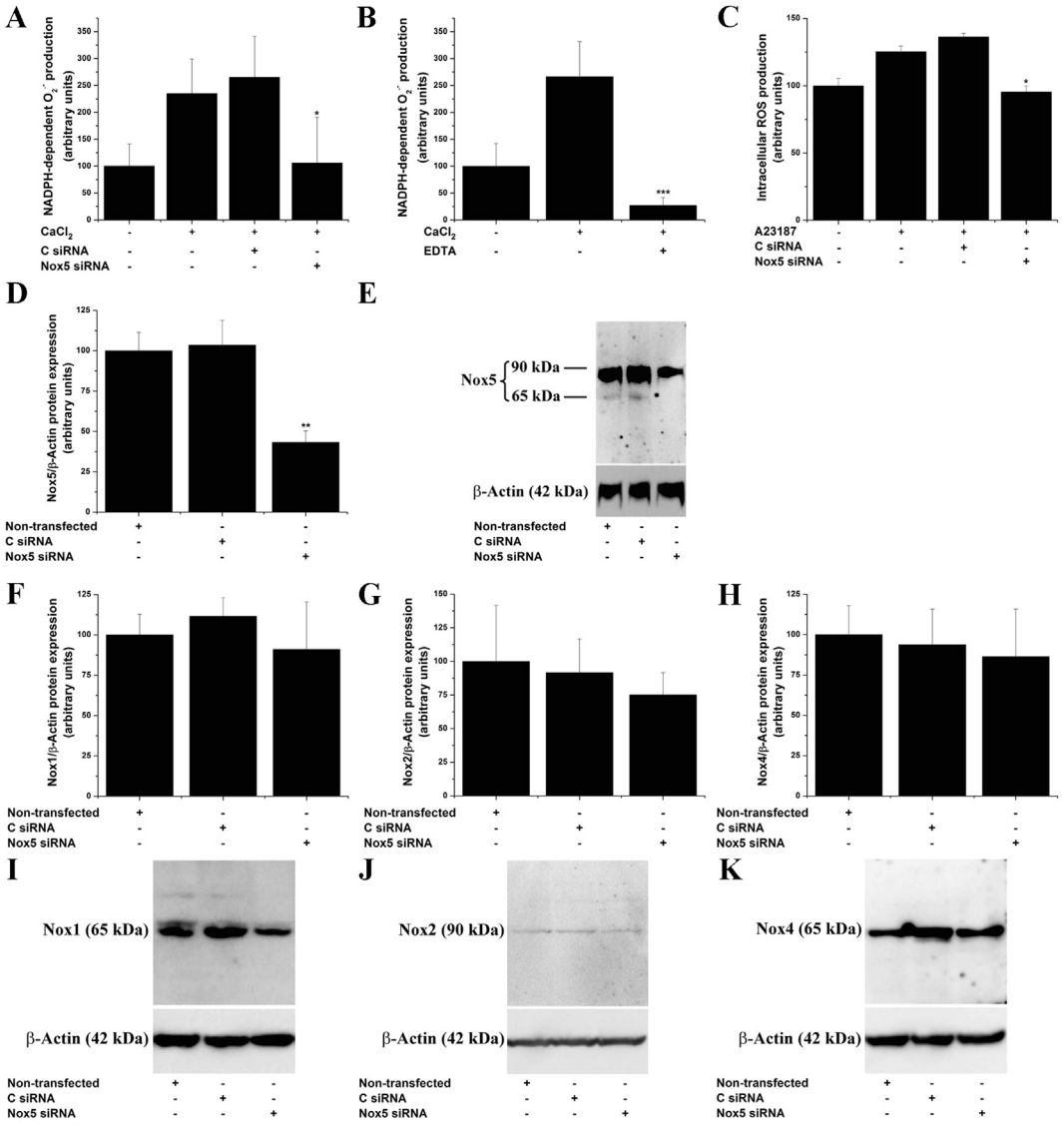

Fig. 2. Analysis of Nox5-derived ROS in Mac. (A) Assessment of Ca^{2+}- independent/dependent Nox activity in Mac-isolated membrane fractions. (B) The effect of EDTA on Ca^{2+}-dependent Nox activity. (C) Evaluation of intracellular ROS formation by DHE assay in the absence/presence of A23187, a specific Ca^{2+} ionophore. (D) Analysis of siRNA efficiency to induce Nox5 proteins down-regulation. (E) Representative immunoblot depicting the modulation of Nox5 proteins in Mac subjected to siRNA transfection. (F–H) Analysis of Nox1, Nox2, and Nox4 protein expression levels in response to Nox5 silencing. (I–K) Representative immunoblots illustrating the regulation of Nox1, Nox2, and Nox4 proteins upon transfection of the cells with C/Nox5 siRNA; n = 3–5, $^{*}P < 0.05$, $^{**}P < 0.01$. *P*-values were taken in relation to C siRNA-transfected cells.

Western blot (Fig. 2D and E). The protein expression levels of Nox1, Nox2, and Nox4 were not significantly affected by Nox5 silencing (Fig. 2F–K).

3.3. IFNγ and oxLDL up-regulate Nox5 protein level and intracellular Ca^{2+} in Mac

Based on previous reports stating that Nox5 expression is up-regulated by pro-inflammatory factors [13,14,23,24], we questioned whether such a regulatory mechanism exists in Mac treated with pro-atherogenic factors. Quiescent THP-1 Mac were exposed (24 h) to vehicle (control), IFNγ (5–100 ng/ml) or oxLDL (5–100 μg/ml) in serum-free medium and the expression of Nox5 proteins was assessed by Western blot. IFNγ increased dose-dependently (≈1.5- to 1.75-fold) the expression of both Nox5-related proteins of ≈90 kDa and ≈65 kDa (Fig. 3A and C). Similar patterns of increased expression of Nox5 were detected in oxLDL-treated cells (Fig. 3B and D). Representative immunoblots showing the dose–response regulation of Nox5 protein expression levels by IFNγ or oxLDL are depicted in Fig. 3E and F. Note that serum deprivation led to a significant down-regulation of the ≈90 kDa Nox5 protein level (control) whereas IFNγ or oxLDL gradually up-regulated its expression.

We questioned next whether the elevation in Nox5 proteins is associated with changes in $[Ca^{2+}]_i$, a contributor to Nox5 activity. To address this issue, Fura 2-AM assay was employed to monitor the $[Ca^{2+}]_i$ in IFNγ- or oxLDL-exposed Mac. Treatment of Mac with increasing concentrations of IFNγ (5–100 ng/ml) for 10 min resulted initially in a slight increase in $[Ca^{2+}]_i$. Maximal effects were achieved in the range of 25–100 ng/ml IFNγ (≈1.25-fold). Exposure of Mac to oxLDL (5–100 μg/ml) led to a steady oxLDL-dependent increase of $[Ca^{2+}]_i$ in the range of 5–25 μg/ml oxLDL (≈1.15- to 1.5-fold) and to a robust mobilization of intracellular Ca^{2+} in the range of 50–100 μg/ml oxLDL (≈2- to 2.5-fold) compared to control level (Fig. 3G).

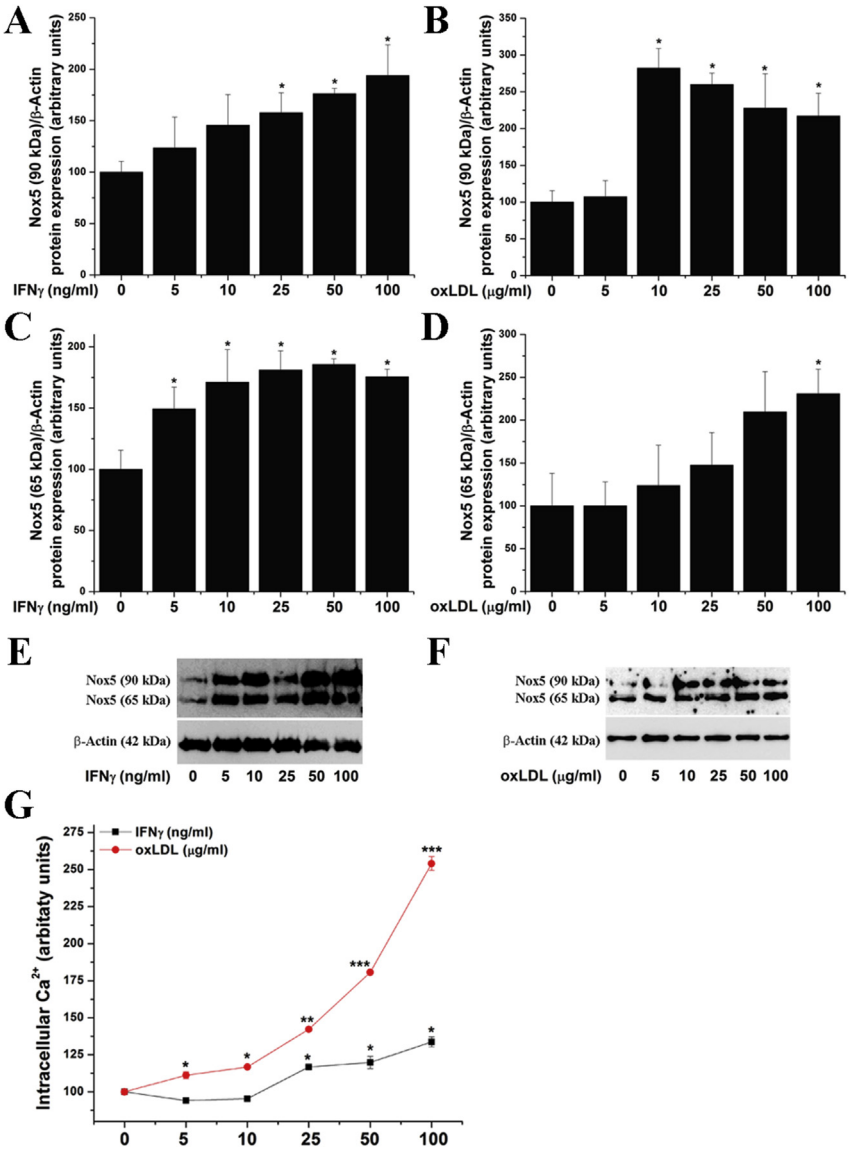

Fig. 3. IFNγ and oxLDL up-regulate Nox5 protein expression and intracellular concentration of Ca^{2+} in Mac. (A,C) As a function of concentration, IFNγ induces up-regulation of both, ≈90 kDa and ≈65 kDa Nox5 protein levels. (B,D) Similarly, oxLDL-exposed Mac exhibit up-regulation ≈90 kDa and ≈65 kDa Nox5 protein, compared to cells exposed to vehicle, only. (E,F) Representative immunoblots depicting Nox5 proteins in Mac incubated with increasing concentrations of IFNγ or oxLDL; n = 5, $^{*}P < 0.05$. *P*-values were taken in relation to vehicle-exposed Mac. (G) IFNγ and oxLDL augment the concentration of intracellular Ca^{2+} in Mac; n = 4, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. *P*-values were taken in relation to vehicle-exposed cells.

3.4. Nox5 protein is present in macrophage-rich area within human atherosclerotic lesions

To examine whether Nox5 protein is expressed in lesional Mac, IHC analyses were performed using human carotid atherosclerotic plaques. Nox5 protein was barely detectable in non-atherosclerotic sections derived from the superior thyroid arteries (Fig. 4A). In contrast, a robust staining (expression) of Nox5 was present in the αSMA-positive regions. Notably, IHC staining of atherosclerotic lesions revealed that Nox5 is also localized within the lipid-rich areas expressing the lysosomal membrane glycoprotein CD68, a pan-macrophage marker (Fig. 4B). No IHC reaction was detected when primary antibody was omitted (No Ab I) or replaced with normal rabbit IgG (isotype control). No apparent vascular staining of Nox5 was detected in mouse aortic cryosections (Fig. 4C). A representative angiographic image depicting the relative extent and localization of atherosclerotic plaques is shown in Fig. 4D. Western blot analysis demonstrated a significant up-regulation of Nox5 protein in the atherosclerotic versus non-atherosclerotic arterial samples and confirmed the expression of the ≈90 kDa and ≈65 kDa proteins in human carotid atherosclerotic plaques (Fig. 4E and F).

4. Discussion

Evidence exists that Nox5 expression and activity are up-regulated in atherosclerosis [25]. Reportedly, various Nox5 isoforms mediate ECs and SMCs proliferation [17,26]. Despite of numerous accumulating data, the function of vascular Nox5 is arguable. Within the cardiovascular system, Nox5 has been detected in ECs, SMCs and cardiac myocytes [17,27]. Hitherto, reports on the expression, regulation, and function of Nox5 in Mon and Mon-derived Mac are missing.

We hypothesized and designed experiments to find out whether Nox5, if present, could function as an additional source of ROS in

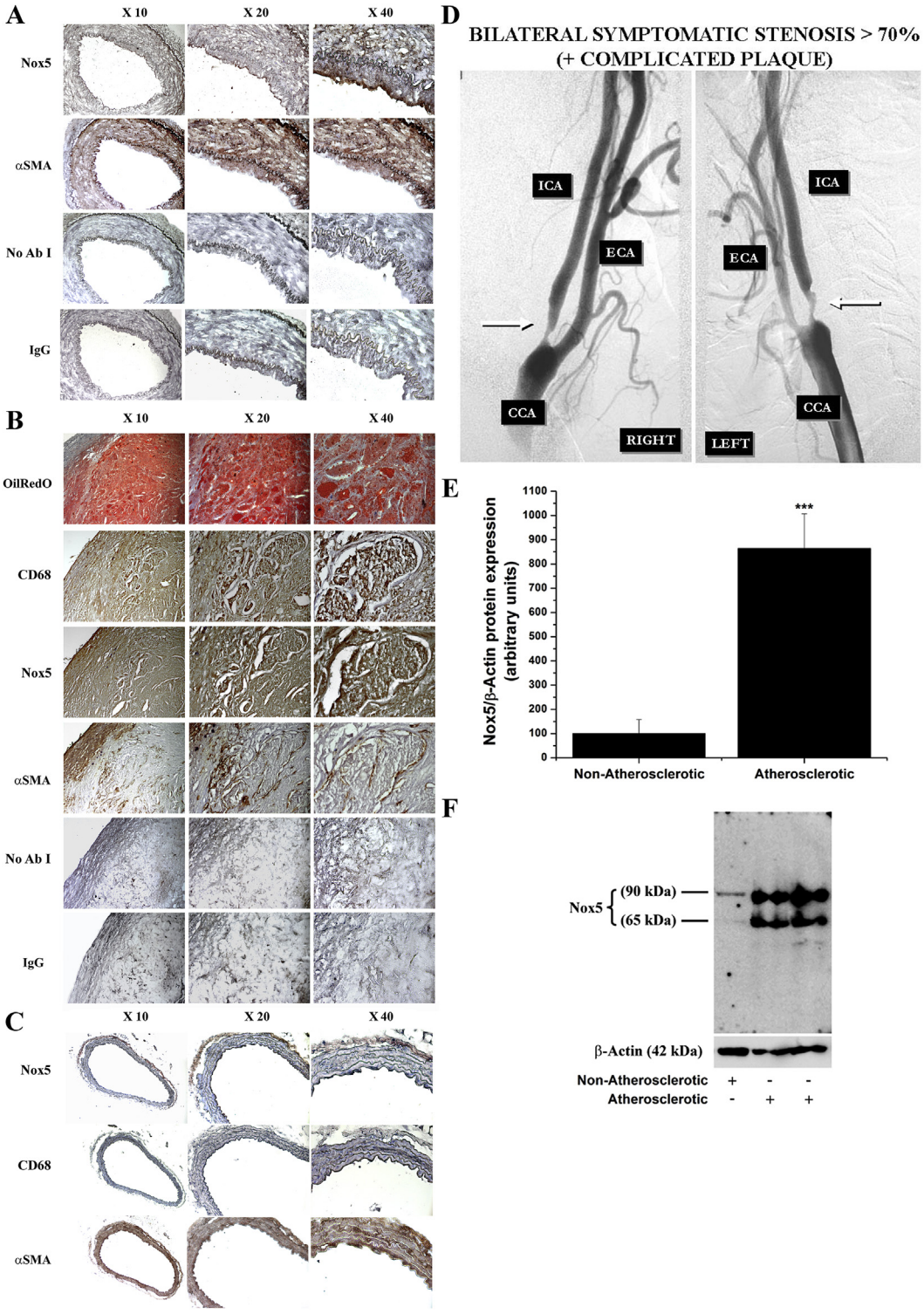

Fig. 4. Immunohistochemical localization of Nox5. (A) Detection of Nox5 protein in non-atherosclerotic fragments. (B) The extent of lipid deposition was assessed by Oil Red O staining. Lesional Mac were localized by means of CD68 expression, a pan-macrophage marker, SMCs were identified by αSMA immunostaining. (C) Assessment of Nox5 antibody specificity employing mouse aortic sections. "No primary antibody" (No Ab I) and rabbit IgG were used as negative controls. Representative images of at least 5 independent experiments. (D) Angiographic image of both carotid bifurcations (RIGHT and LEFT) in the same patient, demonstrating severe stenosis (white arrows) of the origin of the internal carotid arteries (ICA). ECA: External carotid artery. CCA: common carotid artery. (E) Analysis of Nox5 protein level in non-atherosclerotic versus atherosclerotic human arteries ($n = 5$, $^{***}P < 0.001$). (F) Representative immunoblot depicting the expression of Nox5 variants in homogenates derived from human non-atherosclerotic and atherosclerotic arteries.

these cells. The main findings of this study are: (1) Nox5α and Nox5β transcription variants, and Nox5 proteins (of ≈90 kDa and ≈65 kDa) are constitutively expressed in human Mon and Mac (THP-1 monocytic cell line and in human primary $CD14^+$ Mon); (2) Nox5 contributes to ROS formation in human Mac; (3) IFNγ and oxLDL induce the up-regulation of Nox5 protein and elevation of $[Ca^{2+}]_i$; (4) Nox5 is present in the lipid- and $CD68^+$ Mac-rich area within human carotid atherosclerotic plaques.

Nox enzymes regulate important redox-sensitive signalling pathways involved in Mon priming, adhesion, migration [28,29] and Mac death in response to oxLDL [5]. The search for the existence of Nox5 in human Mon was done on human monocytic THP-1 cell line and $CD14^+$ Mon. RT-PCR analysis revealed that the Nox5 mRNA is present in both, the cell line and the primary Mon. To explore which transcription variants are expressed in Mon, the previously described primer sets were employed [17]. The gene expression assays confirmed the expression of Nox5α, Nox5β, Nox5δ, and Nox5γ transcription variants in SMCs as reported in Ref. [17]. Conversely, in the case of human Mon, the major splice variants that are actively transcribed are only Nox5α and Nox5β, as revealed by gene expression assays.

Expression of Nox5 protein was investigated using a polyclonal antibody designed to detect all Nox5 subtypes. The results confirmed the expression of Nox5 protein in SMCs at the predicted molecular weight of ≈90 kDa. Two protein bands of ≈90 kDa and ≈65 kDa were detected in both THP-1 cells and primary $CD14^+$ Mon. In agreement to the predicted molecular weight, one can hypothesize that the ≈90 kDa band corresponds to Nox5 subtypes carrying the Ca^{2+}-binding domain, whereas the ≈65 kDa protein band corresponds to the Nox5ε isoform. The Nox5 protein level was significantly down-regulated in THP-1 Mac transfected with Nox5 siRNA; these results confirm further the specificity of the immunoblotting detection of Nox5. Collectively, the data revealed that Mon (THP-1 and primary $CD14^+$ Mon) display a similar pattern of Nox5 gene and protein expression.

Since Mac are important sources of ROS, the ability of Nox5 to produce ROS in Mac was questioned. The results showed that Nox activity and intracellular the ROS formation was significantly reduced in Nox5 siRNA-transfected cells suggesting that Nox5 plays a role in mediating Ca^{2+}-induced ROS formation.

The physiological relevance of Nox5-derived ROS is not entirely understood. BelAiba et al. showed that all Nox5 variants are functionally active in human ECs [17]. In contrast, Pandey et al. demonstrated that only the Nox5α and Nox5β are able to produce ROS whereas Nox5δ, Nox5γ, and Nox5ε are inactive. In addition, it has been suggested that Nox5ε may act as a negative regulator of Nox5 [9]. Our experiments demonstrated that human Mon express Nox5α and Nox5β, and possibly Nox5ε. Still, the role of each Nox5 subtype in Mon and Mac requires further investigation.

Nox5 expression and activity are induced in vascular cells by many pro-inflammatory factors [13,14,23,24]. Consequently, the expression of Nox5 has been investigated in Mac exposed to IFNγ or oxLDL. We found that both IFNγ and oxLDL induced a significant but different up-regulation of Nox5 protein level. Collectively, these results indicate the existence of a similar pattern of Nox5 regulation in various cell types in response to pro-atherogenic stimuli.

Since Nox5 activity is Ca^{2+}-dependent, we have investigated the impact of IFNγ and oxLDL on $[Ca^{2+}]_i$. The results showed that both IFNγ and oxLDL led to a significant up-regulation of $[Ca^{2+}]_i$. We presume that IFNγ and oxLDL-triggered elevation of $[Ca^{2+}]_i$ associated with increased expression of Nox5 proteins in Mac may represent a mechanism for Nox5 activation in atherosclerosis.

To ascertain the potential implication of Nox5 in atherosclerosis, IHC analysis using specific antibodies against Nox5, CD68 or αSMA was applied to human carotid atherosclerotic plaques. Our data confirmed the previous observations regarding the low level of Nox5 in non-atherosclerotic arteries and the robust expression of Nox5 protein within the αSMA-positive areas [25]. In addition, we found a widespread Nox5 staining in the lipid-rich atherosclerotic regions expressing CD68, a pan-macrophage marker.

The localization of Nox5 in the $CD68^+$ Mac-rich area within human carotid artery atherosclerotic lesions as well as up-regulation of Nox5 protein and $[Ca^{2+}]_i$ in IFNγ/oxLDL-exposed Mac point to Mac-derived Nox5 as a potential source of ROS in atherosclerosis. To the best of our knowledge, this is the first report providing evidence on the expression and regulation of Nox5 in human Mon and Mac. This knowledge may be applicable to numerous maladies (e.g., diabetes, obesity, hypertension, neurodegenerative diseases, and cancer), since controlling oxidative stress ought to have a benefic knock-on effect on these pathologies.

Conflict of interest

The authors declare that there is no conflict of interest in this work.

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