Blockade of Ets-1 attenuates epidermal growth factor-dependent collagen loss in human carotid plaque smooth muscle cells

Velidi H. Rao, Vikrant Rai, Samantha Stoupa, and Devendra K. Agrawal

Center for Clinical and Translational Science, Creighton University School of Medicine, Omaha, Nebraska

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Rao VH, Rai V, Stoupa S, Agrawal DK. Blockade of Ets-1 attenuates epidermal growth factor-dependent collagen loss in human carotid plaque smooth muscle cells. Am J Physiol Heart Circ Physiol 309: H1075–H1086, 2015. First published August 7, 2015; doi:10.1152/ajpheart.00378.2015.—Although degradation of extracellular matrix by matrix metalloproteinases (MMPs) is thought to be involved in symptomatic (S) carotid plaques in atherosclerosis, the mechanisms of MMP expression are poorly understood. Here, we demonstrate that collagen loss in vascular smooth vessel cells (VSMCs) isolated from S plaques was induced by epidermal growth factor (EGF) through the activation of p38-MAPK and JNK-MAPK pathways. Inhibitors of p38-MAPK and JNK-MAPK signaling pathways downregulated the expression of MMP-1 and MMP-9. In addition, we examined whether v-ets erythroblastosis virus E26 oncogene homologue 1 (Ets-1), an important regulator of different genes, is involved in destabilizing S plaques in patients with carotid stenosis. We demonstrate that EGF induces Ets-1 expression and decreases interstitial and basement membrane collagen in vascular smooth muscle cells (VSMCs) from patients with carotid stenosis. Increased expression of MMP-1 and -9 and decreased collagen mRNA transcripts were also found in Ets-1-overexpressed VSMCs. Transfection with both dominant-negatives form of Ets-1 and small interfering RNA blocked EGF-induced MMP-1 and -9 expressions and increased the mRNA transcripts for collagen I (α1) and collagen III (α1) in S compared with asymptomatic (AS) carotid plaques. Inhibitors of p38-MAPK (SB202190) and JNK-MAPK (SP600125) signaling pathways decreased the expression of Ets-1, MMP-1, and MMP-9 and increased collagen type I and III expression in EGF-treated VSMCs. This study provides a mechanistic insight into the role of Ets-1 in the plaque destabilization in patients with carotid stenosis involving p38-MAPK and JNK signaling pathways.

carotid plaque; collagen types I and III; epidermal growth factor; matrix metalloproteinases; v-ets erythroblastosis virus E26 oncogene homologue 1; vascular smooth muscle cells

NEW & NOTEWORTHY

This study provides a mechanistic insight into the role of Ets-1 regulated EGF induced collagen loss involving p38-MAPK and JNK signaling pathways in human carotid plaques with carotid stenosis. Selective blockade of Ets-1 and EGF receptor may be a novel strategy and promising target for treating unstable and vulnerable plaques.

ATHEROSCLEROSIS is a complex disease with coronary thrombus leading to stroke and is the major cause of morbidity and mortality throughout the world (8, 24). The matrix accumulation and degradation in the extracellular matrix (ECM) may determine the outcome of plaque stability (1). Proteases produced by the cellular components of the plaque are thought to be mainly responsible for thinning of the plaque cap and the development of myocardial infarction and stroke. Inflammatory cytokines (tumor necrosis factor-α and interleukin-1) and growth factors [epidermal growth factor (EGF), transforming growth factor-β, and platelet-derived growth factor] secreted by the atherosclerotic plaques are involved in several diseases (25, 32). EGF receptor (EGFR) is a member of the protein tyrosine kinase family and plays an important role in various cellular processes such as proliferation, migration, and apoptosis (57). Many signaling cascades involving phosphatidylinositol 3-kinase-Akt (PI3K/Akt) pathway and mitogen-activated protein kinase (MAPK) are involved in cell migration and apoptosis in various pathophysiological processes (27). However, the involvement of p38-MAPK and JNK-MAPK in EGF-mediated collagen loss in atherosclerotic plaques is poorly understood. Recently, we reported that EGF induces collagen loss involving EGFR and matrix metalloproteinases (MMPs), especially MMP-1 and MMP-9, in isolated vascular smooth muscle cells (VSMCs) from vulnerable carotid plaques (45).

Many cells including endothelial cells, VSMCs, and fibroblasts express v-ets erythroblastosis virus E26 oncogene homologue 1 (Ets-1) and regulate several biological processes such as angiogenesis, development, and apoptosis (37, 49, 60). The Ets-1 proto-oncogene protein is a member of the Ets family of transcription factors with a highly conserved Ets domain (9). Several MMPs contain Ets binding sequence in their functional promoter region (20, 26). However, the Ets-1 regulation of MMP-1 and MMP-9 expression in EGF-treated VSMCs isolated from carotid plaques is not known.

The regulatory elements of different transcription factors, including activator protein 1, NF-κB, and Ets, are present in the promoter region of various MMP genes. In this present study, the regulatory role of Ets-1 on EGF-induced MMP-1 and MMP-9 expression was studied using inhibitors of different signaling pathways in VSMCs isolated from human carotid plaques. We report for the first time that both p38-MAPK and JNK-MAPK signaling pathways are required for the upregulation of Ets-1, MMP-1, and MMP-9 expression induced by EGF, leading to plaque instability in VSMCs derived from the carotid plaques of asymptomatic (AS) and symptomatic (S) patients.

MATERIALS AND METHODS

Human tissue collection. The experimental research protocol, including the collection of the biopsy tissues, and all tissue acquisition procedures were approved by the Institutional Review Board of Creighton University and exempted the research protocol since the carotid endarterectomy specimens were truly anonymized. The patients in both AS and S groups were aged between 50–75 yr and included both men and women of any ethnic origin. These patients were deemed appropriate based on American Heart...
Association criteria that define the risk-to-benefit ratio in AS and S carotid disease. The carotid plaques from patients undergoing carotid endarterectomy were collected. The classification of the carotid plaques as unstable or stable from S and AS patients, respectively, was accordingly made to the criteria previously reported by us (8, 45). Briefly, the carotid plaques were clinically classified by the surgeon as S from the history and clinical evaluation of the patients, including frequent plaque rupture, fibrous cap thinning, and fibrous cap foam-cell infiltration compared with those in AS patients. The symptoms also included hemispheric transient ischemic attacks, amaurosis fugax, or stroke (8, 45). The information on the categorization (S and AS plaque) of carotid stenosis was provided to laboratory investigators in an anonymous manner. The specimens were collected fresh in the University of Wisconsin solution and transported to the laboratory where all procedures were carried out under sterile conditions.

Isolation of VSMCs. An established method developed in this laboratory was used to isolate VSMCs from carotid plaques (8, 18, 45). Briefly, the medial layer was homogenized, digested with trypsin, followed by digestion with collagenase type IA from *Clostridium histolyticum* (C2674, Sigma, St. Louis, MO) and the pellet was suspended in smooth muscle cell medium (ScienCell, Carlsbad, CA). The cells from the second to fifth passage were used. The phenotype and the homogeneity of isolated smooth vessel cells were confirmed by positive staining for smooth muscle α-actin and caldesmon, as previously reported (8, 18, 45).

Cell culture and treatment protocol. VSMCs at preconfluence were incubated in serum-free medium containing 10 ng/ml EGF for

### Table 1. Primer sequences

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
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<tbody>
<tr>
<td>MMP-1</td>
<td>5′-TTC AAC TCT GAC GTT GAT CCC AGA-3′</td>
<td>5′-ACT GCA CAT GTG TCC TGG AGG TGC-3′</td>
</tr>
<tr>
<td>MMP-9</td>
<td>5′-ATT GCA CGC AGG ACC GTT ACT-3′</td>
<td>5′-CAG TTT GTA TGC GGC AAA CTT GGT-3′</td>
</tr>
<tr>
<td>EGF receptor</td>
<td>5′-AGG AAC AAG CTT GCT GTG ACC ACT-3′</td>
<td>5′-TTT GCA GTG GAA GCC TTT AAG CAG-3′</td>
</tr>
<tr>
<td>COL I (α1)</td>
<td>5′-CAA TGG TGG CCT TGG TGC TGG TTT-3′</td>
<td>5′-CAG TGG GTT GTA CGA TGG CTT-3′</td>
</tr>
<tr>
<td>COL III (α1)</td>
<td>5′-TAT CGA ACA CGC AAG GCT GTG AGA-3′</td>
<td>5′-GCC CAA GTG CCA CAC CAA ATT CTT-3′</td>
</tr>
<tr>
<td>Ets-1</td>
<td>5′-GGA GGA CGA GTG GTG GTA AA-3′</td>
<td>5′-AAC TGG CAT AGC TGG ATT GG-3′</td>
</tr>
<tr>
<td>Ets-2</td>
<td>5′-GAG CCA CCG TCC CGA CCA AG-3′</td>
<td>5′-GGT GCC TGG CCG TGG ATG GT-3′</td>
</tr>
<tr>
<td>PEA3</td>
<td>5′-CTG GAC ATT TGC CAT CCT T-3′</td>
<td>5′-AAC TGG CAT AGC TGG ATT TTG-3′</td>
</tr>
<tr>
<td>GAPDH</td>
<td>5′-GGT GAA GAT CCG ACG ATT TGG TAC-3′</td>
<td>5′-GGT CTC CCG TCC TGG AGA ATG GTG ATG GG-3′</td>
</tr>
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MMP, matrix metalloproteinase; Col, collagen; Ets, v-ets erythroblastosis virus E26 oncogene homologue 1; PEA3, polyoma enhancer activator-3.

Fig. 1. EGF induces the expression of v-ets erythroblastosis virus E26 oncogene homologue 1 (Ets-1) mRNA transcripts in vascular smooth vessel cells (VSMCs) (A, N = 5) and tissues (B, N = 5). Treatment with EGF (10 ng/ml) increased the Ets-1 mRNA expression in both asymptomatic (AS) and symptomatic (S) groups (C, N = 3). The mRNA expression of Ets-2 and polyoma enhancer activator-3 (PEA3) in VSMCs are given in D and E. AG1478, an inhibitor of EGF receptor (EGFR), downregulated the Ets-1 mRNA expression (F, N = 3). Results were expressed as fold change in S compared with AS. Data are shown as means ± SD. *P < 0.05. N = 5 for A, B, D, and E. S + E, symptomatic + EGF.
24 h. The activation of EGFR was confirmed by treating VSMCs with an inhibitor of EGFR, and AG1478 (AG Scientific, San Diego, CA) at 15 μM in the presence or absence of EGF.

**Immunofluorescence microscopy.** Cryosections (5 μm) from both S and AS carotid plaques were subjected to immunofluorescence microscopy, as described earlier (44, 45) using rabbit polyclonal antibodies for Ets-1, collagen (Col) I (α1), Col III (α1), and Col IV (α1) (1:100 dilution, Santa Cruz Biotechnology). Antibodies to phosphorylated (p)-p38-MAPK and p-JNK were obtained from Cell Signaling Technology (Beverly, MA) and used at 1:250 dilution. The primers for different genes were obtained from Integrated DNA Technologies (Coralville, IA). The PCR-cycling conditions included 5 min at 95°C for initial denaturation, 40 cycles of 30 s at 55–60°C (depending on the primer annealing temperatures), and 30 s at 72°C, followed by melting curve analysis. Fold expression of mRNA transcripts relative to controls was determined after normalizing to GAPDH. The oligonucleotide primer sequences for MMP-1, MMP-9, Col I (α1), Col III (α1), Ets-1, Ets-2, and polyoma enhancer activator-3 genes are given in Table 1. Fold expression relative to controls was determined after normalizing to GAPDH expression.

**Analysis of signaling pathways in EGF-induced Ets-1, MMP-9, and MMP-1 expression.** Preconfluent VSMCs were treated in serum-free medium for 1 h with inhibitors of ERK1/2 (U0126), p38-MAPK (SB203580), JNK (SP600125), and PI3K (LY294002), followed by treatment with EGF at 10 ng/ml for 24 h. The mRNA expression of Ets-1, MMP-1, and MMP-9 was analyzed by qPCR.

**Transient transfection experiments with Ets-1.** A full-length human Ets-1 and dominant-negative (DN) Ets-1 constructs cloned into pcDNA3.1 (−) neovector were kind gifts from Dr. Stephen Lee (Department of Cellular and Molecular Medicine, University of Ottawa, Ottawa, Ontario, Canada). VSMCs isolated from both AS and S plaques were grown to 60–80% confluence without antibiotics. For transient transfection, the cells were transfected using Lipofectamine 2000 (Life Technologies, Carlsbad, CA) according to the manufacturer’s protocol with either mammalian expression constructs for Ets-1 or DN Ets-1 (17). For small interfering RNA (siRNA) experiments, cells were transfected with siRNA-targeting Ets-1 or with a scrambled control siRNA using Lipofectamine 2000 (Invitrogen). After 24 h of transfection, the cells were stimulated with or without EGF (10 ng/ml) and used for qPCR to quantify mRNA expression of Col I (α1), Col III (α1), MMP-1, and MMP-9 and GAPDH genes using the primers previously described (45).

**Statistical analysis.** All data are presented as means ± SD from three independent experiments using carotid endarterectomy tissues from individual patients (N = 3–5 in each group). Statistical analysis

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**Fig. 2.** Immunofluorescence staining of Ets-1 in carotid plaques. The immunoreactivity of Ets-1 was greater in S (D) compared with AS (A) plaques and are in agreement with mRNA transcripts of Ets-1 in VSMCs (Fig. 1, A and B). B and E: nuclear staining with 4’,6-diamidino-2-phenylindole (DAPI). C and F: merged images of Alexa 596 with DAPI. Quantification of average immunofluorescence intensity in carotid plaque tissue sections (G) demonstrated increased Ets-1 immunoreactivity in S compared with AS. This is a representative image from 5 individual tissues in each group. Data are shown as means ± SD. *P < 0.05; N = 5.
was performed using Student’s t-test between the tissues or VSMCs from AS and S plaques. Multiple group comparison was performed using ANOVA. A P value of <0.05 was considered significant.

RESULTS

Increased expression of Ets-1 in S human carotid plaques and VSMCs. We analyzed the expression of Ets-1 in both tissue extracts and isolated VSMCs. The mRNA expression of Ets-1 was significantly increased in both isolated VSMCs and tissue carotid plaques from S patients (Fig. 1, A and B). The Ets-1 mRNA expression was increased more in tissues than in VSMCs. However, the expression of Ets-2 (Fig. 1D) and polyomavirus enhancer activator-3 (Fig. 1E) did not significantly change between AS and S groups. The Ets-1 immunofluorescence was greater in tissue sections (Fig. 2D) and VSMCs (Fig. 3M) of S compared with AS plaques. Quantification of immunofluorescence in carotid plaque tissue sections (Fig. 2G) and VMSCs (Fig. 3S) demonstrated increased Ets-1 immunoreactivity in S compared with AS plaques, and EGF treatment further enhanced the intensity of immunoreactivity of Ets-1 in VSMCs (Fig. 3P). These results confirm increased expression of Ets-1 mRNA transcripts in both tissues and VSMCs.

EGF-induced expression of Ets-1 mRNA expression is mediated by EGFR in VSMCs. Changes in the mRNA transcripts of Ets-1 were examined in VSMCs isolated from both AS and S plaques with or without EGF treatment (10 ng/ml) for 24 h. The mRNA transcripts for Ets-1 were significantly increased in response to EGF treatment in VSMCs from both AS and S groups (Fig. 1, C and F).

EGFR mediates the expression of MMP-1 and -9 and Ets-1 in VSMCs. We studied whether changes induced by EGF acts through its receptor (EGFR). To demonstrate the involvement of EGFR, the VSMCs isolated from both AS and S plaques were treated with EGF alone or in combination with AG1478, an inhibitor of EGFR, at 15 μM for 24 h in serum-free medium. There was no effect of AG1478 by itself on the mRNA expression of Ets-1 in either AS or S VSMCs. However, the increased mRNA expression of Ets-1 in both AS and S VSMCs treated with EGF was significantly decreased with AG1478 (Fig. 1F).

Fig. 3. Immunofluorescence staining of Ets-1 in VSMCs. The immunoreactivity of Ets-1 was greater in S (M) compared with AS (D) VSMCs. EGF treatment further increased the immunostaining of Ets-1 in both the groups (G and P). Results from immunofluorescence are in agreement with mRNA transcripts of Ets-1 in VSMCs (Fig. 1, A and B). A and J: isotype negative controls (−ve). B, E, H, K, N, and Q: nuclear staining with DAPI. C, F, I, L, O, and R: merged images of Alexa 596 and DAPI. Quantification of average immunofluorescence intensity of Ets-1 (S) also confirmed increased Ets-1 expression (Fig. 1, A and C). This is a representative image from 5 individual tissues in each group. Data are shown as means ± SD. *P < 0.05; N = 5.
EGF induces Ets-1 expression through p38-MAPK and JNK-MAPK pathways. We have demonstrated (45) increased expression of MMP-1 and MMP-9 in EGF-treated VSMCs isolated from S carotid plaques compared with AS; however, the signaling pathways involved are not investigated. Therefore, we looked at the specific roles of p38-MAPK, PI3K/Akt, JNK, and ERK1/2 signaling pathways in the regulation of MMP-1, MMP-9, and Ets-1 mRNA expression using specific inhibitors (Fig. 4, A–D). VSMCs isolated from carotid plaques from AS and S were treated with selective inhibitors (SB202190 to inhibit p38-MAPK, LY294002 to inhibit PI3K/Akt, SP600125 to inhibit JNK, and U0126 to inhibit ERK1/2) with or without EGF (10 ng/ml) for 24 h. In the untreated VSMCs, there was increased expression of EGFR in S-plaque VSMCs compared with AS-plaque VSMCs (Fig. 4D). Inhibitors of p38-MAPK and JNK-MAPK significantly attenuated the EGF-stimulated mRNA transcripts for Ets-1 (Fig. 4A), MMP-1 (Fig. 4B), MMP-9 (Fig. 4C), and EGFR (Fig. 4D) in both AS and S. The PI3K inhibitor LY294002 induced a partial decrease against the changes induced by EGF. However, blockade of ERK with U0126 had no effect on the EGF-induced changes in either Ets-1 or MMPs (Fig. 4).

Increased p-p38-MAPK and p-JNK-MAPK immunostaining in tissue sections from S plaques. Activation of p38-MAPK and JNK pathways in EGF-treated VSMCs was also demonstrated by immunofluorescence staining (Fig. 5). The immunoreactivity of p-p38-MAPK (Fig. 5D) and p-JNK-MAPK (Fig. 5J) was greater in tissue sections of S compared with AS plaques. In addition, quantification of the fluorescence intensity from the images revealed a significant increase in p38-MAPK (Fig. 5M) and JNK-MAPK (Fig. 5N) in S compared with AS plaques. These immunofluorescence studies further support the findings on the expression of EGF-induced expression of Ets-1, MMP-1, and MMP-9 in VSMCs (Fig. 4).

Ets-1 regulates EGF-mediated expression of MMP-1, MMP-9, Col I (α1), and Col III (α1) gene expression. The promoter region of different MMPs and collagens is regulated by a number of transcription factors including Ets-1. We have demonstrated that EGF induces the expression of MMP-1, MMP-9, and type I and III collagen genes that are associated
with the instability of atherosclerotic plaques (45). To evaluate the exact mechanism, VSMCs were transfected either with control pcDNA or Ets-1 pcDNA and then treated with EGF (10 ng/ml) for 24 h. The mRNA transcripts for MMP-1 (Fig. 6A), MMP-9 (Fig. 6B), Col I (α1) (Fig. 6C), and Col III (α1) (Fig. 6D) were significantly increased in VSMCs isolated from S compared with AS patients, and mRNA transcripts for these molecules were further increased with EGF treatment. The immunofluorescence staining and quantification of the sections from plaques further demonstrated increased protein expression of collagen types I, III, and IV in AS compared with S (Fig. 7, A, G, M, and S–U).

DN Ets-1 pcDNA and siRNA downregulate the expression of Col I (α1), and Col III (α1) mRNA transcripts in EGF-treated VSMCs. Treatment with EGF significantly increased the MMP-1 and MMP-9 mRNA transcripts (Fig. 8, B and C; and Fig. 9, B and C) and decreased the expression of both Col I (α1) and Col III (α1) mRNA transcripts in VSMCs from S (Fig. 8, D and E; and Fig. 9, D and E). Transfection with DN Ets-1 or siRNA Ets-1 significantly attenuated the changes induced by EGF (Fig. 8, A–E; and Fig. 9, A–E), whereas transfection with control pcDNA did not influence either the MMP or the collagen genes. These results further confirm the role of Ets-1 in the stabilization of collagen in human carotid plaques.

**DISCUSSION**

Atherosclerosis is a progressive disease of the arterial wall and represents a major growing cause of death and disability worldwide. It is suggested that increased matrix is associated with plaque stability, whereas matrix degradation weakens the fibrous cap and increases its susceptibility to rupture (4, 29, 55). The S carotid plaques are usually associated with ruptured atherosclerotic plaques with thin fibrous cap (23, 24, 42). The risk for atherosclerotic plaque rupture in S plaques is partially mediated by degradation of ECM by MMPs (10, 21, 33, 39). Recently, we have reported increased expression of MMP-1 and MMP-9 and decreased expression of collagen type I and III in VSMCs isolated from S plaques compared with AS plaques, supporting the role of collagen in plaque stability. These results are in agreement with similar studies (45, 50, 53). However, the molecular mechanism of MMP activation in S plaques is poorly understood. Therefore, we investigated the molecular mechanisms of signaling pathways involved in the EGF-mediated activation of MMP-1 and MMP-9, which may be closely related to collagen instability in S plaques.

The ability of EGF to stimulate MMPs depends on the specific signal transduction pathways. MAPKs play a crucial role in cell growth, differentiation, survival, and death (46).
ERK-type MAPKs are activated by mitogenic growth factors and phorbol esters during cell proliferation and differentiation, whereas JNK and p38-MAPK are activated in response to inflammatory cytokines and stress stimuli (11, 46). In various cell types, different signaling pathways (Raf/MEK/ERK, p38-MAPK, and PI3K) were found to upregulate the expression of MMP-1 and MMP-9 (2, 12, 13, 38, 41, 56). In the present study we demonstrate that inhibitors of p38-MAPK and JNK-MAPK downregulated EGF-induced MMP-1 and MMP-9 and increased Col I (10) and Col III (10) mRNA transcripts in EGF-treated VSMCs, suggesting that two or more signaling pathways are involved in the regulation of MMPs in the atherosclerotic plaques. Although ERK-type MAPK activation plays an important role in MMP regulation in different cell types, our results suggest that it is not involved in the regulation of MMPs or collagens in EGF-treated VSMCs.

The promoter regions of MMPs and TIMPs contain activator protein 1 and Ets binding sites and are activated by EGF (22, 36, 41, 58). Ets-1 is one of the mediators of ECM remodeling associated with the regulation of MMPs and hence connected to ECM degradation (43). In response to stimuli, transcription factors containing the Ets binding motif have been implicated in the regulation of MMP genes (3, 5, 6, 15, 19, 34, 52, 59). Ets-1 is activated in VSMCs by a variety of stimuli including platelet-derived growth factor-BB, interleukin-1, and tumor necrosis factor-α (14, 30, 48, 60). Increased expression of various proteases that degrade the ECM was reported in Ets-1-overexpressing cells (7, 16, 20, 51, 54). However, the involvement of Ets-1 in EGF-induced MMP-1 and -9 expressions in carotid plaque VSMCs are not known. In this study we demonstrate that the expression of Ets-1 was significantly increased in VSMCs isolated from S compared with AS carotid plaques (Fig. 4, A and B). The EGFR inhibitor AG1478 significantly inhibited Ets-1 expression in EGF-treated VSMCs, demonstrating the involvement of EGFR. Transfection of an Ets-1 DNA plasmid further upregulates mRNA transcripts for MMP-1 and MMP-9 in VSMCs isolated from both AS and S carotid plaques, suggesting that Ets-1 regulates both MMP-1 and -9 expressions.

Members of the Ets family that are located in the collagen promoter region are believed to be associated with collagen gene expression (35, 40). Several ECM genes, including type I colla-
Ets-1 (61). It has been shown that elevated expression of recombinant Ets-1 has the potential to suppress the transcription of type I collagen and increase matrix degradation in transforming growth factor-β-treated cells (28, 31, 47). In the present study, the finding of decreased mRNA expression of collagen type I and III in EGF-treated VSMCs suggests that Ets-1 regulates the expression of MMP-1 and MMP-9, leading to collagen loss in S compared with AS. Overexpression of either DN Ets-1 or Ets-1 siRNA suppressed the MMP transcripts and increased both collagen type I and III genes (Fig. 5, B–E), demonstrating the role of Ets-1 in the stability of plaques in VSMCs. Similar results were found with transfection of Ets-1 siRNA.

In summary, our results provide a mechanistic insight that EGF-induced Ets-1 regulates MMP-1, MMP-9, and collagen I and III expression through p38-MAPK and JNK signaling pathways (Fig. 10). The effect of the EGF on downstream signaling molecules is greater in S-plaque VSMCs than the AS-plaque VSMCs. This could be due to either increased EGFR density or increased transmembrane signaling pathways or both in S-plaque VSMCs than in AS-plaque VSMCs. This results in decreased interstitial collagens leading to plaque instability in patients with carotid stenosis. This study also provides the biochemical and molecular evidence that a selective blockade of both Ets-1 and EGFR may be a novel strategy and a promising target for stabilizing the vulnerable atherosclerotic carotid plaques.

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GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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AUTHOR CONTRIBUTIONS


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Fig. 9. Knockdown of Ets-1 gene on the expression of Ets-1, MMP-1, MMP-9, Col I (α1), and Col III (α1) in VSMC-treated EGF. VSMCs were transfected with either Ets-1 small interfering RNA (siRNA) or nonspecific control, followed by treatment with EGF for 24 h. The RNA was subjected to qPCR: Ets-1 (A), MMP-1 (B), MMP-9 (C), Col I (α1) (D), and Col III (α1) (E), and the results were expressed as fold change compared with AS group. Data are shown as means ± SD. *P < 0.05; N = 3.
Fig. 10. A schematic diagram showing the role of EGF-regulated MMP-1 and -9 expressions through Ets-1 activation involving p38 and JNK-MAPK signaling pathways. The upregulated MMPs degrade the fibrillar collagens and basement membrane and destabilize the carotid plaques.


