

Transcriptomic profiling identifies tumor necrosis factor receptor-associated factor (TRAF)-5 expression in human atherosclerotic plaques as a predictor of future cardiovascular events

Mark Colin Gissler^a, Xin Gu^a, Floor B.H. van der Zalm^b, Hauke Horstmann^a, Juana Dominguez^a, Patrick M. Siegel^a, Daniela Stallmann^a, Constantin von zur Muehlen^a, Timoteo Marchini^a, Yvonne Döring^{c,d,e,f}, Matthias Heinig^g, Dirk Westermann^a, Michal Mokry^{b,1}, Dennis Wolf^{a,*,1}

^a Department of Cardiology and Angiology, University Heart Center Freiburg, Bad Krozingen, Faculty of Medicine, University of Freiburg, Germany

^b Central Diagnostic Laboratory, University Medical Center Utrecht, Utrecht, Netherlands

^c Swiss Cardiovascular Center, Division of Angiology, Inselspital, Bern University Hospital, University of Bern, Bern, Switzerland

^d Institute for Cardiovascular Prevention (IPEK), Ludwig-Maximilians-Universität, Munich, Germany

^e Department for BioMedical Research (DBMR), Bern University Hospital, University of Bern, Bern, Switzerland

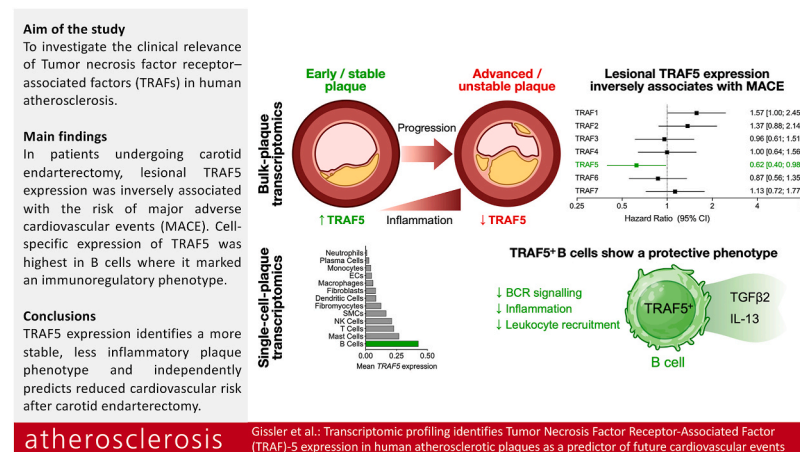
^f DZHK (German Center for Cardiovascular Research), Partner Site Munich Heart Alliance, Munich, Germany

^g Institute of Computational Biology, German Research Center for Environmental Health, Helmholtz Zentrum München, Neuherberg, Germany

HIGHLIGHTS

- Tumor necrosis factor receptor-associated factors (TRAFs) are cytoplasmic adapter proteins that regulate immune cell signaling, inflammation, and atherosclerosis in rodent models.
- The clinical relevance of TRAFs in human atherosclerosis is unknown.
- In human atherosclerotic plaques, low TRAF5 expression associates with advanced disease stages and predicts cardiovascular outcomes after carotid endarterectomy.
- Elevated TRAF5 expression identifies a stable, less inflammatory plaque phenotype and a likely protective, immunoregulatory B cell state.

GRAPHICAL ABSTRACT



* Corresponding author. Cardiology and Angiology, University Heart Center, Faculty of Medicine, Hugstetterstrasse 55, 79106, Freiburg, Germany.
E-mail address: dennis.wolf@uniklinik-freiburg.de (D. Wolf).

¹ M.M. and D.W. share senior authorship.

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ABSTRACT

Aim of the study: Tumor necrosis factor receptor-associated factors (TRAFs) are cytoplasmic adapter proteins that regulate immune cell signaling downstream of TNF, interleukin-1, and Toll-like receptors. Although preclinical data implicate TRAFs in atherogenesis, their clinical relevance in human disease remains unknown. We aimed to characterize TRAF1–7 expression in human atherosclerotic plaques and determine associations with plaque phenotype, cardiovascular outcomes and cellular functions.

Main findings: TRAF1–7 expression was compared between early and advanced carotid plaques using bulk transcriptomics. Prognostic relevance was assessed in 616 carotid endarterectomy specimens from the Athero-Express Biobank using multivariable Cox regression adjusted for age, sex, diabetes, diastolic blood pressure, eGFR, and peripheral arterial disease. Major adverse cardiovascular events (MACE) comprised nonfatal myocardial infarction, nonfatal stroke, and cardiovascular death within 3 years. Potential TRAF5-dependent immune cell mechanisms were explored by single-cell RNA sequencing (scRNA-seq) of 259,493 cells from 79 human plaques. Among all TRAFs, only TRAF5 was significantly downregulated in advanced lesions. Higher lesional TRAF5 expression independently predicted lower risk for MACE (HR 0.62; 95% CI 0.40–0.98). TRAF5 was lowest in fibro-inflammatory and unstable plaques. scRNA-seq identified B cells as the predominant TRAF5-expressing population. TRAF5⁺ B cells showed downregulation of pro-inflammatory pathways and upregulation of immunoregulatory mediators such as TGF- β 2 and IL-13. Gene-set enrichment analyses confirmed attenuated inflammatory activation and B cell receptor signaling in TRAF5⁺ B cells.

Conclusions: TRAF5 expression identifies a stable, less inflammatory plaque phenotype and independently predicts reduced cardiovascular risk after carotid endarterectomy positioning it as a potential biomarker for personalized cardiovascular risk stratification.

1. Letter

Atherosclerotic cardiovascular disease (CVD) is driven by chronic inflammation within the vessel wall [1]. Accumulating evidence indicates that atherosclerotic plaques are populated by immune cells that engage in crosstalk with one another and with stromal cells. Induction of pro-inflammatory signalling pathways in lesional immune cells drives disease progression [2]. The mechanistic role of inflammation has been validated clinically by large outcome trials demonstrating reduced cardiovascular events following inhibition of pro-inflammatory key cytokines, such as IL-1 β . However, broad and long-term suppression of inflammation is at the expense of serious adverse events, including infections, thereby limiting the applicability of anti-inflammatory therapies. It remains a substantial unmet need to identify inflammatory master regulators that enable more nuanced immunomodulation and personalized therapies in ASCVD.

Tumor necrosis factor receptor-associated factors (TRAFs) are cytoplasmic adapter proteins that integrate and regulate receptor-mediated signaling downstream of TNF receptors (TNFRs), interleukin-1 receptors (IL1Rs), and Toll-like receptors (TLRs), thereby modulating cellular survival, function, and activity in various immune cells [3]. Preclinical studies have implicated TRAFs in regulating vascular inflammation and atherosclerosis in mice [3]. However, the clinical relevance of TRAFs remains unknown. Therefore, we performed a comprehensive analysis of TRAF expression in human atherosclerotic plaques, leveraging bulk transcriptomic data and single-cell RNA sequencing (scRNA-seq). First, we analyzed TRAF1–7 expression in 9 early and 8 advanced human carotid plaques. We observed that TRAF5 expression was lower in advanced than in early lesions, whereas the other TRAFs remained unregulated (Fig. 1A). Protein expression of TRAF5 in sections of human carotid plaques was confirmed by immunohistochemistry (Fig. 1B). Next, we analyzed TRAF expression in 616 human plaques obtained from carotid endarterectomy specimens in independent cohort (Athero-Express Biobank) and tested its association with the occurrence of major adverse cardiovascular events (MACE), defined as a composite of nonfatal myocardial infarction, stroke, and cardiovascular death, within 3 years after surgery. Notably, only TRAF5 expression levels, but not that of other TRAFs, was inversely associated with the risk of MACE in a multivariable Cox proportional hazards regression model adjusted for age, sex, diabetes, diastolic tension, eGFR, and peripheral arterial occlusive disease (Fig. 1C). Among molecular plaque phenotypes, TRAF5 expression was lowest in fibro-inflammatory plaques (Fig. 1D).

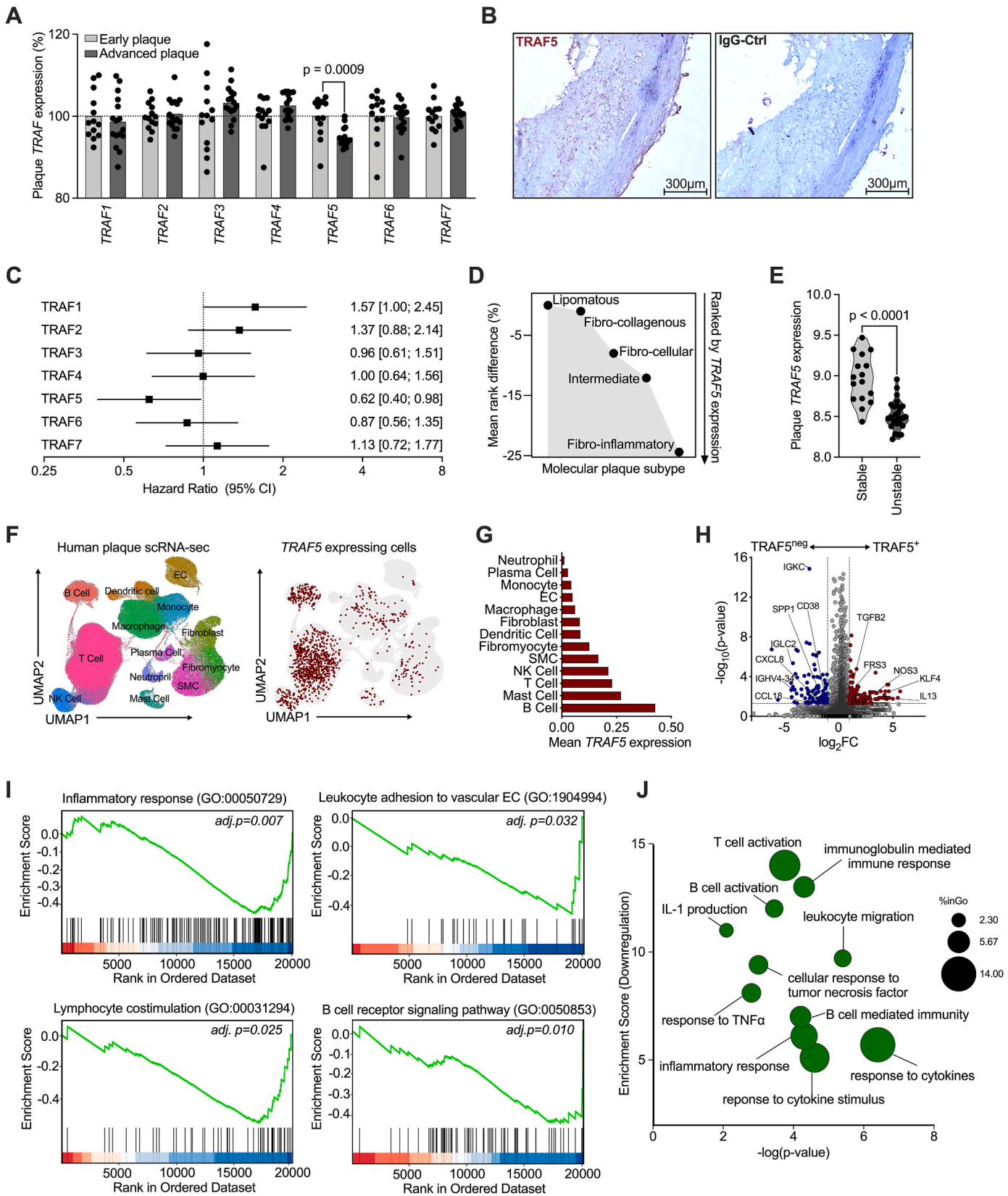
Consistently, TRAF5 mRNA expression was lower in unstable plaques (Fig. 1E). Together, these data show a robust association between TRAF5 expression and plaque vulnerability and outcomes in human atherosclerotic plaques.

Because TRAF5 transcripts may originate from different cell types, we next sought to define potential cell-specific mechanisms of TRAF5 using scRNA-seq of 259,493 cells from 79 human atherosclerotic plaques. TRAF5 was expressed by various cell types, including stromal cells and leukocytes. We found the highest expression of TRAF5 in B cells (Fig. 1F–G) where it was expressed across all B cell subsets with the highest expression found in APC-like B2 cells; in contrast, TRAF5 was barely expressed in plasma cells (Supplemental Figure).

In TRAF5⁺ B cells, 258 genes were regulated differently compared to TRAF5-non-expressing B cells. Several proinflammatory pathways, including those implicated in Interleukin (IL)-1 production, B cell activation and response to tumor necrosis factor signaling, were lower in TRAF5⁺ B cells. Likewise, transcripts encoding multiple chemokines instrumental in leukocyte recruitment to atherosclerotic lesions, such as CXC motif chemokine ligand (CXCL)8 and Chemokine CC motif ligand (CCL)18, were expressed at a lower level in TRAF5⁺ B cells. Expression of immunoglobulin kappa constant (IGKC) and immunoglobulin Lambda Constant 2 (IGLC2) was attenuated in TRAF5⁺ B cells. In contrast, immunoregulatory mediators were enriched in TRAF5⁺ B cells, including transforming growth factor- β 2 (TGFB2) and Interleukin-13 (IL-13) (Fig. 1H). Overall, these findings argue for an association of TRAF5 with a less inflamed plaque phenotype characterized by modulated inflammatory responses, BCR signalling, and cell activation and recruitment as confirmed by GSEA and pathways analysis (Fig. 1I–J).

Finally, a transcriptional signature of TRAF5⁺ B cells derived from scRNA-seq was associated with a less atheromatous plaque phenotype and a lower risk of MACE over a follow-up period of 3 years after carotid endarterectomy in the Athero-Express bulk transcriptomic plaque cohort, supporting the notion that TRAF5 defines a protective and immunoregulatory B cell state in human atherosclerosis (Fig. 2).

In this study, we identify TRAF5 as a transcriptional hallmark of atherosclerosis progression that defines a likely protective B cell state. We and others have previously established that TRAFs are critically involved in immune cell functions and serve as key regulators of inflammatory cardiometabolic diseases, including atherosclerosis [3,8,9]. Here, we show that TRAF5 gene expression is associated with favorable cardiovascular outcomes. Our data are consistent with a protective role of TRAF5 in human atherosclerosis and implicate inflammation-driven



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Fig. 1. Expression of *TRAF5* in human atherosclerotic plaques correlates inversely with adverse cardiovascular outcomes and inflammation in B cells. Expression of *TRAF1-7* in advanced atherosclerotic plaques (thin or thick fibrous cap atheroma) compared with early lesions (pathological intimal thickening and intimal xanthoma, data from GSE28829). For genes with multiple probes, the one with the highest mean expression values was selected for analysis (A). Representative immunohistochemistry for anti-*TRAF5* and an IgG control in a human carotid atherosclerotic plaque (B). Forest plot showing adjusted Hazard Ratio (HR) and 95% CI (error bars) for occurrence of major adverse cardiovascular events during 3 years of follow-up in 616 patients after carotid endarterectomy from the Athero-Express Biobank. HR was calculated by Cox proportional hazards regression analysis in patients with a dichotomized threshold for plaque *TRAF1-7* expression with adjustment for age, sex, diastolic tension, diabetes mellitus, eGFR and peripheral occlusive arterial disease (C). Ranked mean expression of *TRAF5* across transcriptomic-based molecular plaque subtypes [4] in 616 carotid endarterectomy samples (D). *TRAF5* gene expression in stable vs. unstable atherosclerotic lesions (data from GSE163154) (E). Uniform manifold approximation and projection (UMAP) visualization of scRNA-seq profiles of immune cell from a meta data set of 79 pooled human plaques generated as previously described [5] (left) and an overlay of *TRAF5* expressing cells (right) (F). Average expression of *TRAF5* per cell type (G). Volcano plot of differentially expressed transcripts in *TRAF5*⁺ vs *TRAF5*-negative B cells adjusted with Benjamin-Hochberg correction for multiple testing (H). Gene set enrichment analysis for GO-pathways GO:00050729, GO:1904994, GO: 00031294, and GO:0050853 (I). Bubble plots displaying significantly down-regulated pathways in *TRAF5*⁺ B cells from human atherosclerotic plaques (J). Statistical significance was assessed by a Mann-Whitney test (A), multivariable logistic regression (D), or an unpaired *t*-test (E).

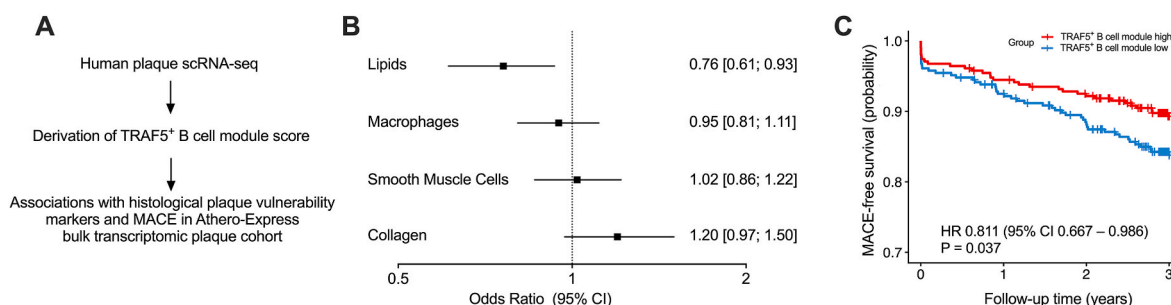


Fig. 2. Lesional expression of the *TRAF5*⁺ B cell gene signature correlates inversely with plaque lipid content and adverse cardiovascular outcomes Schematic of the analytical workflow. A gene module score for *TRAF5*⁺ B cells consisting of the top 15 genes ranked by fold change that showed *TRAF5*⁺ B cell-specific expression in human atherosclerotic plaque single-cell RNA-sequencing [5] was derived by k-means clustering as previously described [6] (A). Associations of lesional expression of the *TRAF5*⁺ B cell module with plaque vulnerability markers defined by histology (lipids defined as >10% lesional fat content, macrophages defined by moderate or heavy CD68 staining, smooth muscle cells defined by absent or minor α -actin staining, collagen defined by absent or minor picrosirius red staining) [7] (B). Associations of lesional *TRAF5*⁺ B cell module expression with major adverse cardiovascular events in 616 patients after carotid endarterectomy during 3 years of follow-up. Kaplan-Meier curves show MACE-free survival probability in patients dichotomized by median of *TRAF5*⁺ B cell module score expression. HR was calculated by cox regression analysis with the *TRAF5*⁺ B cell module score as a continuous variable and adjustments for age, sex, diastolic tension, diabetes mellitus, eGFR and peripheral occlusive arterial disease (C). Statistical significance was assessed by logistic regression (B) and multivariable adjusted cox regression (C). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

plaque destabilization as a potential mechanism. *TRAF5* expression has been detected on multiple cell types, including endothelial cells, adipocytes, and immune cells. Our scRNA-seq analysis identified B cells as the population with the highest *TRAF5* expression in human atherosclerotic plaques. Resolving stage-dependent *TRAF5* expression within plaque B cells remains to be assessed by future single-cell studies with systematic histological grading of the sequenced tissue. Since *TRAF5* is not a canonical component of the BCR signalosome, *TRAF5* most likely modulates B cell activity through promoting crosstalk of BCR co-receptors, such as TLR and TNF receptor family members. This observation is particularly relevant given the emerging role of B cells as important modulators of vascular inflammation in atherosclerosis. Our findings extend this framework by identifying *TRAF5* as a possible modulator of B cell function in human atherosclerosis.

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Declaration of competing interest

None.

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The graphical abstract was created with schematics from BioRender.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.atherosclerosis.2026.121886>.

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