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SHORT COMMUNICATION

TSPAN4 and migrasomes in atherosclerosis regression correlated to myocardial infarction and pan-cancer progression

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ABSTRACT

The migrasomes formation is mediated by the assembly of micron-scale tetraspanin macrodomains and the recruitment of tetraspanin 4 (TSPAN4). However, the physiological functions of TSPAN4 on migrasomes are less known. The TSPAN4 expression in macrophages in single-cell sequencing data, GEO datasets and TCGA database were determined. TSPAN4 expression was highly associated with atherosclerosis regression-related macrophages, intraplaque hemorrhage and ruptured plaques. TSPAN4 expression was upregulated in spontaneous MI and inducible MI mice model. Besides, TSPAN4 expression was highly correlated with tumor-associated macrophages. The study provided a critical role of TSPAN4 aberrant expression in the progression of atherosclerosis and pan-cancer, and the intervention of TSPAN4 and migrasomes may save dying patients' lives and improve their prognosis.

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TSPAN4; migrasomes; atherosclerosis; myocardial infarction; pan-cancer; progression; regression

Introduction

Migrasomes formation is mediated by the assembly of micron-scale tetraspanin macrodomains and the recruitment of tetraspanin 4 (TSPAN4) [1]. Yu Li's recent works demonstrated that proteins and mRNAs can be laterally transferred to other cells via migrasomes [2,3]. However, whether this lateral transfer of material occurs *in vivo* and whether it has any physiological functions are less known. Nevertheless, it is our belief that evolution does not invent something that only works *in vitro*. Therefore, the investigation of TSPAN4 functions may help understand migrasomes in diseases and provide novel targets for treatment.

Tetraspanin 4 (TSPAN4) interacts with histamine H4 receptor (H4R) in transfected cells [4]. The podocytes release the 'injury-related' migrasomes [5]. Therefore, the investigation of TSPAN4 functions may help understand migrasomes in diseases and provide novel targets for treatment.

Lin JD et al. reported that single-cell analysis of fate-mapped macrophages revealed heterogeneity during atherosclerosis progression and regression [6]. In this study, TSPAN4 expression in macrophages was explored in single-cell sequencing, GEO datasets, TCGA pan-cancer database and MI mice model, which may be a potential diagnostic and prognostic target for patients with cardiovascular diseases and pan-cancer.

Materials and methods

RNA-seq data and data processing

Single-cell sequencing data about TSPAN4 expression in macrophages in atherosclerosis were used from Single Cell Portal (Study: SCP491, https://singlecell.broadinstitute.org/).

Using the keywords 'atherosclerosis' in 'Homo sapiens', GSE163154 and GSE41571 from the Gene Expression Omnibus (GEO) database was investigated, processed with log2 transformation for normalization and analyzed using the limma package in R. The RNA-sequencing of macrophages was based on the Affymetrix Human Genome U133A Array.

The Cancer Genome Atlas (TCGA) database and the Genotype-Tissue Expression (GTEx) database by UCSC XENA (https://portal.gdc.cancer.gov/). The gene expression data from TCGA pan-cancer, including unpaired samples and paired samples, were analyzed

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using Xiantao website tool (www.xiantao.love). The immune infiltration analysis and the correlation between pan-cancer and tumor-associated macrophages were applied.

MI mice model

Adult experimental C57Bl/6 J, HDL receptor$^{\pm}$ and ApoE$^{-/-}$ mice male mice were purchased from Charles River (Beijing, China). Mice were maintained in an SPF environment with free access to food and water and a 12/12 light–dark cycle. Protocols were approved by Nankai University and Nankai University Affiliated Third Center Hospital.

Spontaneous MI mice model group HDL receptor$^{\pm}$ ApoE$^{-/-}$ mice, control group ApoE$^{-/-}$ mice and treatment group HDL receptor$^{\pm}$ ApoE$^{-/-}$ + Probucol (ip, 8 mg/kg) was used to investigate the effect of TSPAN4 expression on cardiac function after spontaneous MI formation.

Myocardial infarction

MI was induced in adult mice (10–11 weeks). Through inhalation of isoflurane (1.5–2%, MSS-3, England), left coronary artery was ligated, and infarction was considered successful following an ST elevation on electrocardiogram. Sham-operated animals underwent the same procedure without any coronary artery ligation.

ELISA

The left ventricular samples in spontaneous MI mice, control group and treatment group were collected at 31d and 43d after birth. The left ventricular samples in infarcted area and remote area in inducible MI and SHAM mice were collected at 1 h, 4 h,1d, 2d, 1 w and 8 w after operation.

For further validations, left ventricular sample level of TSPAN4 and TSPAN7 expressions were performed with mouse TSPAN4 ELISA kit (abx551579, Abbexa Ltd, UK) and mouse TSPAN7 ELISA kit (OKEH05774, Aviva Systems Biology, United States).

Statistical analysis

All the data are presented as the mean ± SD. Statistical analyses were performed using SPSS 23.0. The Shapiro-Wilk normality test and Welch's t test (two groups) were used. A value of $P < 0.05$ was considered statistically significant.

Results

Using the single-cell sequencing analysis, TSPAN4 expression was highly associated with atherosclerosis regression-related macrophages, which showed stem-like properties and may persist in a proliferating self-renewal state in plaques, while there was little correlation to another migrasomes-related gene TSPAN7 (Figure 1a). To explore the effects of TSPAN4 expression, GSE163154 and GSE41571 were download and analyzed, and demonstrated TSPAN4 rather than TSPAN7 was highly expressed in the presence of intraplaque hemorrhage or ruptured plaques (Figure 1b, c), suggesting TSPAN4 expression may be a novel potential target of the regression macrophages response to atherosclerosis progression.

Myocardial infarction (MI) promotes atherosclerosis and thus MI itself progression through the release of progenitor cells and hematopoietic stem cells from the bone marrow niche. Monocytes/macrophages contribute to all stages of atherosclerosis and myocardial infarction. Intervention of monocytes/macrophages impedes atherosclerosis progression. Using a spontaneous MI mice model, the TSPAN4 expression was upregulated at 31d and 43d after birth in double knockout (HDL receptor and ApoE knockout) mice compared to ApoE$^{-/-}$ mice, which can be rescued by utilizing Probucol treatment (Figure 1d). Using an inducible MI mice model, TSPAN4 was lowly expressed at 4 h after MI and became highly expressed at 1d and 1 w, which may be due to the mitochondrial quality control from CD169 macrophages and tissue-resident macrophages (Figure 1e).

MI can also epigenetically reprogram Ly6C^{high} monocytes in the bone marrow, which were increasingly recruited to breast cancer microenvironment and promoted MI-induced early-stage breast cancer progression and increasing breast cancer patients' mortality and morbidity. Atherosclerosis can impact cancer progression due to cholesterol and calcium metabolism, illustrating the links between atherosclerosis and cancer metastasis. Therefore, the crosstalk between cardiovascular diseases and tumor progression deserves to be investigated, which may be potential targets to inhibit tumor progression and improve prognosis. In this study, TSPAN4 expression in pan-cancer was explored using TCGA database, and unpaired samples in pan-cancer demonstrated there were significant differences in TSPAN4 expression in 27 cell lines, including Adrenocortical carcinoma (ACC), Bladder Urothelial Carcinoma (BLCA), Breast invasive carcinoma (BRCA), Cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), Cholangiocarcinoma (CHOL), Colon adenocarcinoma (COAD), Lymphoid Neoplasm Diffuse

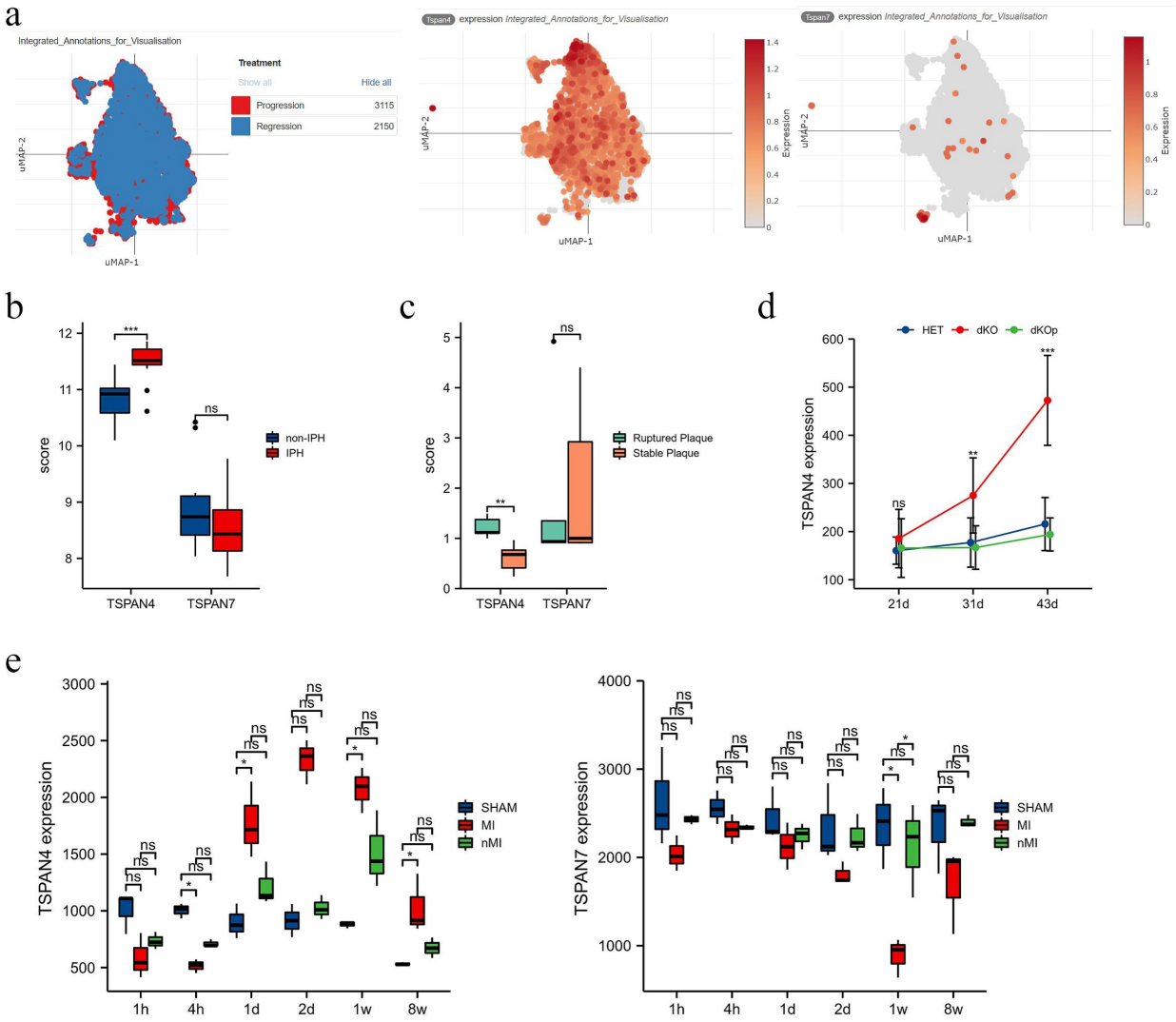

Figure 1. The TSPAN4 expression was correlated to macrophages in atherosclerosis and myocardial infarction. (a) TSPAN4 expression rather than TSPAN7 was highly associated with atherosclerosis regression-related macrophages, which showed stem-like properties and may persist in a proliferating self-renewal state in plaques. (b-c) TSPAN4 rather than TSPAN7 was highly expressed in the presence of intraplaque hemorrhage from GSE163154 (b) or ruptured plaques from GSE41571 (c). IPH, intraplaque hemorrhage. (d) Using spontaneous MI mice model, the TSPAN4 expression was upregulated at 31d and 43d after birth in double knockout (HDL receptor and ApoE knockout) mice compared to $ApoE^{-/-}$ mice, which can be rescue by utilizing Probucol treatment. HET, HDL $receptor^{\pm}$ $ApoE^{-/-}$ mice; dKOp, double knockout mice with Probucol treatment. (e) Using inducible MI mice model, TSPAN4 was lowly expressed at 4 h after MI and became highly expressed at 1d and 1 w. nMI, remote area in MI group; MI, infarction area in MI group.

Large B-cell Lymphoma (DLBC), Esophageal carcinoma (ESCA), GBM, Head and Neck squamous cell carcinoma (HNSC), Kidney Chromophobe (KICH), Kidney renal clear cell carcinoma (KIRC), Kidney renal papillary cell carcinoma (KIRP), Acute Myeloid Leukemia (LAML), Brain Lower Grade Glioma (LGG), Liver hepatocellular carcinoma (LIHC), Lung adenocarcinoma (LUAD), LUSC, Ovarian serous cystadenocarcinoma (OV), Pancreatic adenocarcinoma (PAAD), PRAD, Rectum adenocarcinoma (READ), Skin Cutaneous Melanoma (SKCM), Thyroid carcinoma (THCA), THYM, Uterine Corpus Endometrial Carcinoma (UCEC) and Uveal Melanoma (UCS) (Figure 2a). Macrophages and dendritic cell abundance was significant different between TSPAN4 high- and low-expression groups in BRCA and LGG, which may further show impact on other immune cell abundances (Figure 2b, c). In addition, TSPAN4 expression was highly correlated with tumor-associated macrophages, especially in BLCA, COAD, STAD, LUSC, READ and COADREAD (Figure 2d). A TSPAN4-CD151 fusion gene was highly expressed in a pediatric infratentorial anaplastic ependymoma. The explore of TSPAN4 and migrasomes may treat

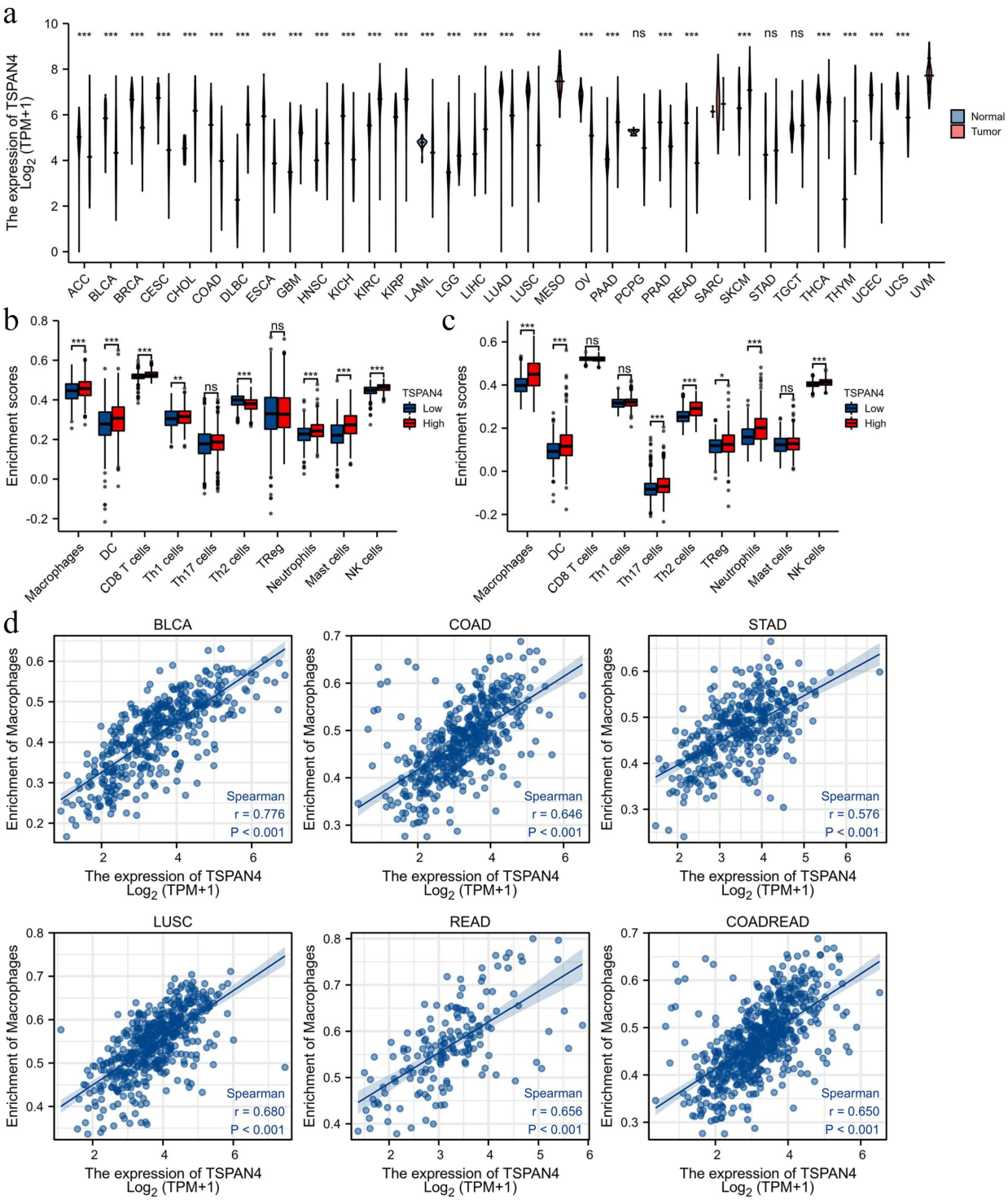

Figure 2. The TSPAN4 expression was correlated to macrophages in and tumor progression. (a) TSPAN4 expression in pan-cancer was explored using TCGA database and unpaired samples in pan-cancer demonstrated there were significant differences in TSPAN4 expression in 27 cell lines. (b-c) Macrophages and dendritic cells abundance were significant different between TSPAN4 high and low expression groups in BRCA and LGG, which may further show impact on other immune cells abundance, such as T reg cells and NK cells. (d) TSPAN4 expression was highly correlated to tumor-associated macrophages, especially in BLCA, COAD, STAD, LUSC, READ and COADREAD patients.

some refractory lesions and help in the surveillance of some acute or chronic diseases progression.

Discussion

TSPANs, such as TSPAN4 and TSPAN7, as well as migrasomes play a critical role in vascular and heart mitochondrial homeostasis [7]. TSPAN4 expression may be a novel potential target of the regression macrophages response to atherosclerosis progression [6]. Migrating cells, such as monocytes and CD169 macrophages, could absorb, eliminate and expel dysfunctional mitochondria in migrasomes to maintain mitochondrial homeostasis and control mitochondrial quality [7,8]. Mitocytosis, a migrasome-mediated mitochondrial quality-control process, may be a novel treatment for heart and vascular under stress. The monocytes/macrophages with the regression status may play a critical role in mitocytosis and diseases progression.

Migrasomes take center stage in disease development [9] and TSPAN4 are essential for migrasome formation. TSPAN4 promoted proliferation and invasion in gastric cancer tissues [10,11] and high TSPAN4 and ELAVL2 expression levels were independent risk factors for poor chemotherapy response in ESCC patients [12]. A TSPAN4-CD151 fusion gene was highly expressed in a pediatric infratentorial anaplastic ependymoma [13].

There are some limitations. Firstly, the quality of collected data relies on the source, which could show an impact on the conclusion. Secondly, the result and conclusions are partly not experimentally validated in the laboratory or clinic. Further studies are still needed to validate in vivo and in vitro.

Conclusion

Overall, this study provided a critical role of TSPAN4 aberrant expression in the progression of atherosclerosis and pan-cancer. Further, experimental investigations are still needed in the laboratory or clinic. If TSPAN4 and migrasomes functions on macrophages in atherosclerosis and the interplay between atherosclerosis and tumor progression, the intervention of TSPAN4 and migrasomes may save dying patients' lives and improve their prognosis. It is worth taking a shot.

Data Availability Statement

The data of pan-cancer analysis can be investigated in TCGA database. (https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga)

Consent for publication

All of the authors have agreed to the submission and publication of this paper.

Ethics approval and consent to participate

The protocol was approved by Nankai University and Nankai University affiliated Tianjin Third Central Hospital and Institute of Radiation Medicine, the Chinese Academy of Medical Sciences.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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