Research Article

Caveolin-1-Knockout Mouse as a Model of Inflammatory Diseases

Elena Codrici,[1] Lucian Albulescu (ID),[1] Ionela Daniela Popescu (ID),[1] Simona Mihai (ID),[1] Ana-Maria Enciu (ID),[1,2] Radu Albulescu,[1,3] Cristiana Tanase (ID),[1,4] and Mihail E. Hinescu (ID)[1,2]

[1]*Victor Babes National Institute of Pathology, 050096 Bucharest, Romania*
[2]*Carol Davila University of Medicine and Pharmacy, 050047 Bucharest, Romania*
[3]*National Institute for Chemical Pharmaceutical R&D, Bucharest, Romania*
[4]*Faculty of Medicine, Titu Maiorescu University, Bucharest, Romania*

Correspondence should be addressed to Ana-Maria Enciu; ana.enciu@ivb.ro and Cristiana Tanase; cristianatp@yahoo.com

Caveolin-1 (CAV1) is the scaffold protein of caveolae, which are minute invaginations of the cell membrane that are involved in endocytosis, cell signaling, and endothelial-mediated inflammation. CAV1 has also been reported to have a dual role as either a tumor suppressor or tumor promoter, depending on the type of cancer. Inflammation is an important player in tumor progression, but the role of caveolin-1 in generating an inflammatory milieu remains poorly characterized. We used a caveolin-1-knockout (CAV1$^{-/-}$) mouse model to assess the inflammatory status via the quantification of the pro- and anti-inflammatory cytokine levels, as well as the ability of circulating lymphocytes to respond to nonspecific stimuli by producing cytokines. Here, we report that the CAV1$^{-/-}$ mice were characterized by a low-grade systemic proinflammatory status, with a moderate increase in the IL-6, TNF-α, and IL-12p70 levels. CAV1$^{-/-}$ circulating lymphocytes were more prone to cytokine production upon nonspecific stimulation than the wild-type lymphocytes. These results show that CAV1 involvement in cell homeostasis is more complex than previously revealed, as it plays a role in the inflammatory process. These findings indicate that the CAV1$^{-/-}$ mouse model could prove to be a useful tool for inflammation-related studies.

1. Introduction

A low-grade chronic inflammatory status is defined as a persistent, mild increase (2 to 4 times greater than normal) in circulating inflammation mediators [1]. Chronic inflammation is associated with a plethora of conditions, including aging (inflammaging) [2], autoimmune diseases [3], cardiovascular pathologies [4, 5], and carcinogenesis [6], as well as the formation and maintenance of a (pre)metastatic niche [7]. Systemic contributors to chronic inflammation are endothelial cells and immune cells, which are also now recognized as important players in tumorigenesis and metastasis [8, 9]. Caveolin-1 (CAV1), the scaffold protein of caveolae, could represent a link between inflammation and tumorigenesis, as it has been associated with both processes. In addition to its scaffolding role, CAV1 acts as a "guardian" by selecting the messages that are sent into cells from the outer

environment. CAV1 recruits various cytoplasmic proteins involved in cell signaling via its caveolin-scaffolding domain. Loss of CAV1 has been associated with a proinflammatory status in senescent endothelial cells [10] and with premature senescence in fibroblasts [11] and was protumorigenic for selected cancers, such as prostate [12] and gastric [13] cancer and glioblastoma [14]. Loss of CAV1 in stromal cells, most notably in the cancer-associated fibroblasts, negatively affected the relapse-free survival of prostate cancer [15], breast cancer [16], and gastric cancer [17] patients. However, whether the lack of CAV1 is directly correlated with chronic inflammation has been insufficiently explored. The involvement of CAV1 in inflammation has only been sporadically addressed, with reports mainly focused on the evaluation of endothelial cells and their role in atherosclerosis [18, 19] and the lung response to sepsis [20–22]. For immune cells involved in the production of inflammatory mediators,

CAV1 has seldom been reported as related to lymphocyte migration [23, 24] and the inhibition of proinflammatory cytokine production in macrophages [18].

The aim of this study was to specifically address the hypothesis that the loss of CAV1 is involved in the pathogeny of the inflammatory response. We examined more than 30 pro- and anti-inflammatory cytokines in the plasma of $CAV1^{-/-}$ mice to assess their inflammatory status, as well as the ability of circulating leukocytes to respond to nonspecific stimuli through the production of cytokines.

2. Materials and Methods

2.1. Mice and Sample Collection. Blood samples were obtained from $CAV1^{-/-}$ mice (CAV1 KO: $CAV1^{tm1Mls}$/J) and $CAV1^{+/+}$ mice (B6129PF2/J), purchased from Jackson Laboratory (Bar Harbor, ME) ($n = 9$). For this study, we used 3-month-old male knockout mice weighing 22 ± 4 g and age-, gender-, and weight-matched control mice. All animal experiments were conducted in accordance with the respective animal welfare guidelines, the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health, and the study was approved by the Institutional Ethical Committee of "Victor Babes" National Institute of Pathology in Bucharest. The adult mice were fed with standard chow and water ad libitum.

2.2. Plasma Preparation. Collection of whole peripheral blood from knockout mice, the STOCK $CAV1^{tm1Mls}$/J and control B6129PF2/J mice, was achieved using vacuum blood tubes (Systems, Becton Dickinson) with heparin (for cell culture/plasma). For plasma extraction, the blood was allowed to clot for at least 30 min at room temperature before centrifugation at 2500 rpm for 10 min. Samples were then aliquoted and stored at $-80°C$ until the multiplex analyses. The plasma samples were collected from mice in a consistent manner, at the same time of the day, between 10:00 a.m. and 11:00 a.m.

2.3. Cell Culture. Whole peripheral blood of both the $CAV1^{-/-}$ and control mice was obtained through retroorbital blood collection and diluted to 5% with RPMI-1640 culture medium (supplemented with 1% antibiotic), in the absence and presence of the polyclonal lymphocyte stimulator, 5 mg/L PHA (Difco, Augsburg, Germany), or 5 mg/L ConA (Difco, Augsburg, Germany) [25]. Whole-blood cell culture was performed in 96-well round bottom plates (Corning CLS3360); after the indicated exposure time to compounds, the plates were centrifuged at $250g$ for 10 minutes and 100 μL supernatants from each sample was collected and stored in 1.8 mL cryo tubes. Cultures were incubated for 24 h and 48 h at 37°C and 5% CO_2 (Shell Lab). Samples were made in triplicate. After 24 h or 48 h of treatment, the supernatant was removed, following centrifugation for 5 min at $250g$. Samples were stored at $-80°C$ until further analysis.

2.4. Assessment of Cytokines by xMAP Analysis. The xMAP array was performed according to the manufacturer's protocols, and the plates were analyzed using a Luminex® 200™ system (Luminex, Austin, TX). Cell culture cytokine levels were determined using the Fluorokine MAP Mouse Base Kit (R&D Systems, USA), with the following analyte-specific bead sets: *GM-CSF, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p70, IL-13, IL-17, CCL2/JE/MCP-1, CXCL1/KC, MIP-2, TNF-α*, and *VEGF*. Plasma cytokine levels were determined using the MILLIPLEX MAP Cytokine Magnetic Bead Panel Kit—*GM-CSF, IL-1β, IL-2, IL-4, IL-6, IL-12p70, IL-13, CXCL1/KC, VEGF*, and *TNF-α* (Merck-Millipore, Billerica, MA, USA). Briefly, the beads were incubated with the samples, buffers, and standards in a 96-well plate at 4°C overnight. All further incubations with the detection antibodies and streptavidin phycoerythrin (SAPE) conjugate were performed at room temperature in the dark with shaking at 800 rpm. Multiplex data acquisition and analysis were performed using STarStation 2.3 (Applied Cytometry Systems, Sheffield, UK) and xPONENT 3.1 software (Millipore, Billerica, MA); the calibration curves were generated with a 5-parameter logistic fit.

2.5. Proteome Profiler™ Antibody Array: A Membrane-Based Assay. Array images were scanned with MicroChemi 4.2 (Berthold Technologies, Chennai, India), and the signal intensity of each spot was analyzed with ImageJ software; the average intensity was calculated by subtracting the average background signal. The cytokine profile assessment, including *CXCL13/BLC/BCA-1, IL-5, M-CSF, C5a, IL-6, CCL2/JE/MCP-1, G-CSF, IL-7, CCL12/MCP-5, GM-CSF, IL-10, CXCL9/MIG, CCL1/I-309, IL-12 p70, CCL3/MIP-1α, CCL11/eotaxin, IL-13, CCL4/MIP-1β, ICAM-1, IL-16, CXCL2/MIP-2, IFNγ, IL-17, CCL5/RANTES, IL-1α, IL-23, CXCL12/SDF-1, IL-1β, IL-27, CCL17/TARC, IL-1ra, CXCL10/IP-10, TIMP-1, IL-2, CXCL11/I-TAC, TNF-α, IL-3, CXCL1/KC, TREM-1*, and *IL-4*, was performed using Mouse Cytokine Array Panel A (R&D Systems Inc., Minneapolis, MN, USA), according to the manufacturer's instructions. Briefly, after the membrane blocking, the plasma samples and detection antibody cocktail were added and incubated overnight at 4°C on a rocking platform shaker. After the unbound proteins were removed by washing, the membranes were incubated with a streptavidin-HRP solution for 30 min at room temperature on a rocking platform and then washed again. Subsequently, protein spots were visualized using the chemiluminescence detection reagents.

2.6. Statistical Analysis. Data were expressed as mean ± standard error of the mean (SEM), and minimum and maximum values were provided when necessary. Duplicate/triplicate samples were used for all specimens, and the average concentrations were used for statistical analysis. Differences between groups were analyzed by two-tailed unpaired Student's t-test. Statistical significance has been indicated as $^*p < 0.05$, $^{**}p < 0.01$, or $^{***}p < 0.001$. Statistical analysis was performed using GraphPad Software.

3. Results

In order to evaluate the inflammatory status of the $CAV1^{-/-}$ mice, we assessed the levels of circulating pro- and anti-inflammatory cytokines and growth factors in plasma. The

pattern of the cytokine and growth factor production in the CAV1$^{-/-}$ mice, compared to the controls, was evaluated by two different multiplex analyses: xMAP technology and proteome profiler analysis. We examined adult animals, for which we confirmed the lack of CAV1 expression, before the onset of any macroscopic tumors (data not shown), in order to discriminate between existing pretumor inflammation and a tumor-driven inflammatory milieu, as these mice have been reported to be prone to tumorigenesis [26].

3.1. Increased Levels of IL-6, TNF-α, and IL-12p70 in the Plasma of CAV1$^{-/-}$ Mice. Overexpression of the plasma levels of the proinflammatory cytokines IL-6 (over a 5-fold change in the KO mice versus control, $p < 0.001$) and TNF-α and IL-12p70 (over a 3-fold change, $p < 0.05$) was detected using the xMAP Luminex 200 platform. IL-4, as well as CXCL1/KC, was also found to be upregulated in the KO mice compared to the control mice (over a 3-fold change and up to 2-fold change, resp.; $p < 0.05$) (Figure 1).

3.2. Proteome Profiler Analysis of Proinflammatory Cytokines and Chemokines in the Plasma of CAV1$^{-/-}$ Mice. In order to establish an overall perspective of the inflammatory status of the CAV1$^{-/-}$ mice, we also performed an array analysis of multiple circulating pro- and anti-inflammatory cytokines and growth factors in plasma. A dot blot assay revealed a relevant pattern for the proinflammatory status (Figures 2(a) and 2(c)). The results showed overexpression of the majority of the cytokines and growth factors in the KO mice, especially for IL-6 (8.6-fold increase in the KO mice versus the control), IL-5 (5.8-fold increase), IL-12p70 (3.8-fold increase), CXCL13/BLC (2.7-fold increase), and G-CSF, CCL2/JE/ MCP-1, TARC, and TIMP-1 (~1.7-fold increase for these 4). By comparing the KO and control groups for cytokine expression, the obtained dot blot values were similar to the outline obtained by the xMAP array analysis.

Proteome profiler analysis confirmed that a set of cytokines, chemokines, and growth factors was overexpressed in the plasma of the CAV1$^{-/-}$ mice compared with that of the control mice, with significant differences for CXCL13/BLC, G-CSF, GM-CSF, CCL1/I-309, IL-3, and CXCL10/IP-10 ($p < 0.05$).

At this point in our study, we concluded that the CAV1$^{-/-}$ mice are characterized by a low-grade systemic proinflammatory status.

3.3. Nonspecific Stimulation of the Lymphocytes of KO Mice with PHA and ConA. CAV1-KO mice were previously reported to show no changes in the percentages of lymphocyte subpopulations [27]; therefore, we used whole peripheral blood to initiate cell cultures and treated them with lymphocyte-targeting stimulants, that is, concanavalin A (ConA) and phytohemagglutinin (PHA). Using whole peripheral blood was reported as a valid method to assess cytokine production [25, 28]. We assessed whether the lymphocytes' response to stimuli is modified by the chronic inflammatory milieu. We found that upon stimulation with ConA or PHA, the production of cytokines/chemokines and growth factors increased, showing that even if these cells

are derived from a medium abundant with proinflammatory cytokines, their response has not reached saturation.

Overall, we noticed an activated status of the CAV1$^{-/-}$ lymphocytes, characterized by an increased response to PHA and ConA stimulation by IL-6, TNF-α, CXCL1/KC, IL-4, and IL12p70, while IL-1β did not show the same trend (Figures 3(a) and 3(f)).

Expression of IL-6 in the CAV1$^{-/-}$ lymphocytes increased at 24 h (7.4-fold compared to the control) and 48 h (17-fold versus the control), following ConA stimulation (Figure 3(a)).

Although TNF-α secretion was not inducible in the control lymphocytes, it increased in the CAV1$^{-/-}$ lymphocytes upon stimulation. The relative increase was 2.8-fold and 7-fold, at 24 h and 48 h, respectively, for ConA stimulation and 5.8-fold and 14-fold, at 24 h and 48 h, respectively, for PHA stimulation (Figure 3(b)). CXCL1/KC was also overexpressed to 1.8-fold and 3.6-fold, at 24 h and 48 h, respectively, for ConA stimulation and to 2.7-fold and 5-fold, at 24 h and 48 h, respectively, for PHA stimulation (Figure 3(c)).

Expression of IL-12p70 in the CAV1$^{-/-}$ lymphocytes increased, but only for the first 24 h after ConA stimulation (3-fold compared with the control), as well as after PHA stimulation (2.5-fold compared with the control) (Figure 3(d)).

IL-4 expression was also elevated in a time-dependent manner upon stimulation in the CAV1$^{-/-}$ mice. Thus, 24 h after ConA stimulation, we observed an increase of almost 7-fold compared to the control, and at 48 h, the increase was 10-fold (Figure 3(e)). Similar to the control lymphocytes, the CAV1$^{-/-}$ lymphocytes were nonresponsive to PHA stimulation.

For IL-1β, the response of the CAV1$^{-/-}$ lymphocytes was time-dependent following ConA stimulation (1.5-fold and 2.5-fold compared to the control at 24 h and 48 h, resp.) (Figure 3(f)).

In conclusion, the circulating levels of the proinflammatory cytokines could generate a chronic inflammatory status; additionally, the lymphocytes of these mice are readily responsive to stimuli, further contributing to the inflammatory status, which can be a useful tool for future studies of the tumor environment or other low-grade chronic inflammatory diseases.

4. Discussion

CAV1 has been repeatedly linked to cancer progression, either as a tumor suppressor, as its absence is associated with a poor prognosis [29] (e.g., aggressive prostate cancer [30, 31], breast cancer [32], and gastric cancer [33]), or as a tumor and metastasis promoter [34–38]. CAV1-KO mice have been used extensively as a model to investigate tumor-related mechanisms, such as tumor growth, pathologic angiogenesis, and tumor invasion [26, 39]. In addition, CAV1-KO cells also activate inflammation-related signaling pathways (e.g., Akt signaling, TLR4 signaling, and ERK signaling), resulting in the production of proinflammatory cytokines, chemokines, and extracellular matrix remodeling enzymes [40]. Less is known about the contribution of CAV1 to the inflammatory milieu. Thus far, studies on CAV1 and inflammation
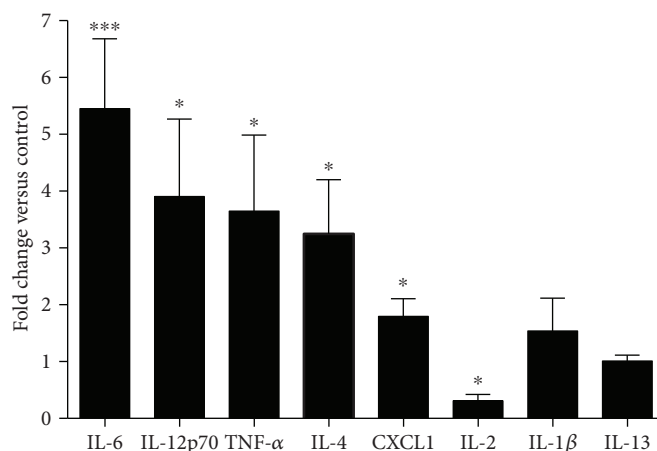
FIGURE 1: Cytokine levels in the plasma of the KO mice ($n = 9$) compared to the controls ($n = 9$). The data represent the average fold modification $\pm$ SEM versus the controls, as determined by the xMAP multiplex assay; molecules have been arranged in order of statistical significance. $^{*}p < 0.05$ and $^{***}p < 0.001$ indicate statistical significance compared with the controls.

have focused on organ-specific effects (lung [41, 42], colon [43, 44], and eye [45]). Additionally, different subsets of leukocytes derived from CAV1-null mice have been analyzed in terms of response to either parasitic [46] or bacterial infection [27]. Also, the ability of lymphocytes from CAV1-null mice to induce a humoral [47] or cytotoxic immune response [48] has been reported. However, studies have not focused on systemic inflammation in the absence of immune triggers.

CAV1 has also been linked with oxidative stress, in a dual manner. On the one hand, CAV1 was shown to be a "critical determinant" of oxidative stress balance. Using the same CAV1-null mouse model, Shiroto et al. found that the redox stress plasma biomarker 8-isoprostane was elevated in the blood of these mice and its involvement in oxidative stress was confirmed by knocking down CAV1 in endothelial cells in an in vitro model [49]. Furthermore, CAV1 was recently pinpointed as a target in cancer-related oxidative stress (reviewed in [50]). The relationship between CAV1, oxidative stress, and inflammation has been best studied in the tumor microenvironment, where loss of CAV1 was reported to lead to oxidative stress and to drive inflammation [51]. Moreover, loss of stromal CAV1 in a tumor model was proposed as a marker of inflammation and a predictor of poor outcome [52].

On the other hand, interesting data emerged about a direct interaction between CAV1 and nuclear factor erythroid 2-related factor 2 (NFE2L2/NRF2) [53], a transcription factor known as "master regulator of oxidative stress response" [54]. In this regard, inhibition of NRF2-mediated signaling by CAV1 activates the p53/senescence pathway [53] and inhibits antioxidant enzymes with antioxidant response element- (ARE-) dependent gene sequences [55].

In our study, we hypothesized that CAV1 has an effect on the systemic inflammation status. We addressed the involvement of CAV1 in enhancing the inflammatory response and creating a low-grade systemic inflammatory milieu. We verified the inflammation status by assessing the pro- and anti-inflammatory cytokine levels in the plasma of CAV1$^{-/-}$ mice compared with that of control mice.

4.1. CAV1$^{-/-}$ Mice Are Characterized by a Low-Grade Systemic Proinflammatory Status.

To investigate whether the absence of CAV1 is related to a low-grade systemic inflammatory milieu, we performed a series of in vitro experiments examining the plasma protein levels of different cytokines in caveolin-1-deficient mice (CAV1$^{-/-}$). To this end, we investigated the expression of circulating proinflammatory (IL-1β, IL-2, IL-6, IL-17, TNF-α, IL-8, IFNγ, CSF, and IL-12p70) and anti-inflammatory (IL-4, IL-10, and IL-13) members of the cytokine family in CAV1-KO mice, before the onset of any clinically overt tumors.

We showed that CAV1$^{-/-}$ mice have enhanced plasma levels of a number of proinflammatory cytokines, including IL-1β, IL-2, IL-6, IL-12, and TNF-α, compared with those in the control mice (Figure 1). Inflammation is an important component of the tumor milieu and of the premetastatic niche, in which IL-6 is an important player [56, 57]. Our results are in agreement with recent findings that link CAV1 expression and IL-6 production. Lee et al. reported that the degradation of CAV1, via the ubiquitin/proteasome pathway, leads to TLR4 activation and the enhanced production of proinflammatory cytokines in bone marrow-derived macrophages [58]. In vitro silencing of CAV1 in mouse keratinocytes has been linked to STAT3 signaling activation, leading to increased expression of IL-6 [59]. Additionally, decreased expression of CAV1 in monocytes from diabetic peripheral neuropathy patients was negatively correlated with IL-6 and TNF-α plasma levels [60]. Taken together, these results support the fact that CAV1 expression is negatively correlated with IL-6 levels.

Weiss et al. also correlated the loss of CAV1 with increased TNF-α and other proinflammatory cytokines in a mouse colitis model [61].
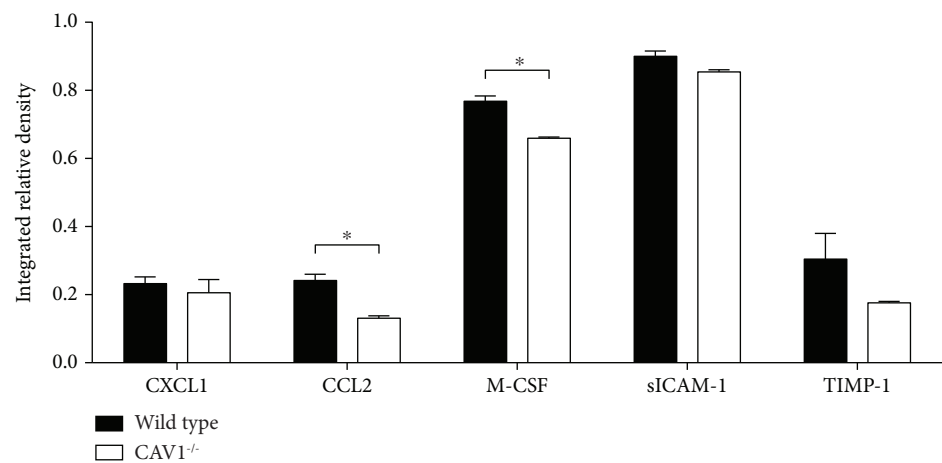
Additionally, we found elevated levels of the anti-inflammatory cytokine IL-4 in the CAV1$^{-/-}$ mice. However, these levels did not increase to the same extent as IL-6, as indicated by the IL-6 : IL-4 ratio of ~1 in the control group and 1.67 in the CAV1$^{-/-}$ mice. One could speculate that IL-4 increases in the CAV1$^{-/-}$ mice as a systemic reaction to
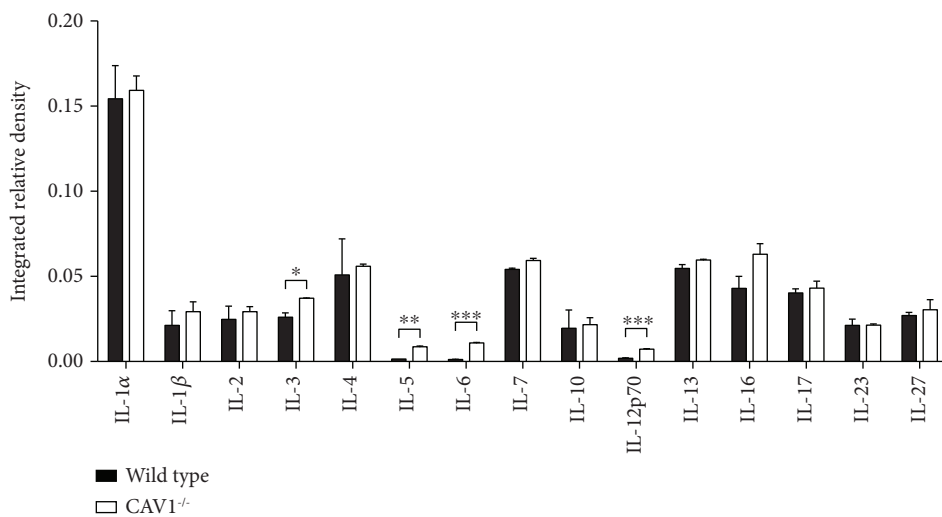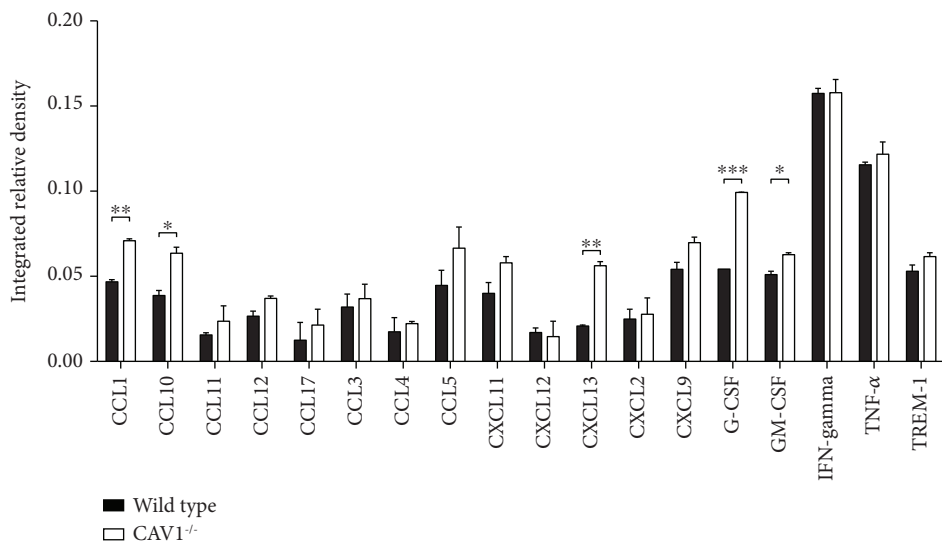
FIGURE 2: Relative expression levels of various mediators of inflammation. Pro- and anti-inflammatory cytokines (a), growth factors (b), and enzymes (c) involved in inflammatory processes were assessed in the plasma of the $CAV1^{-/-}$ and control mice using the Proteome Profiler. The data represent the average of the experiments. $^*p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$.
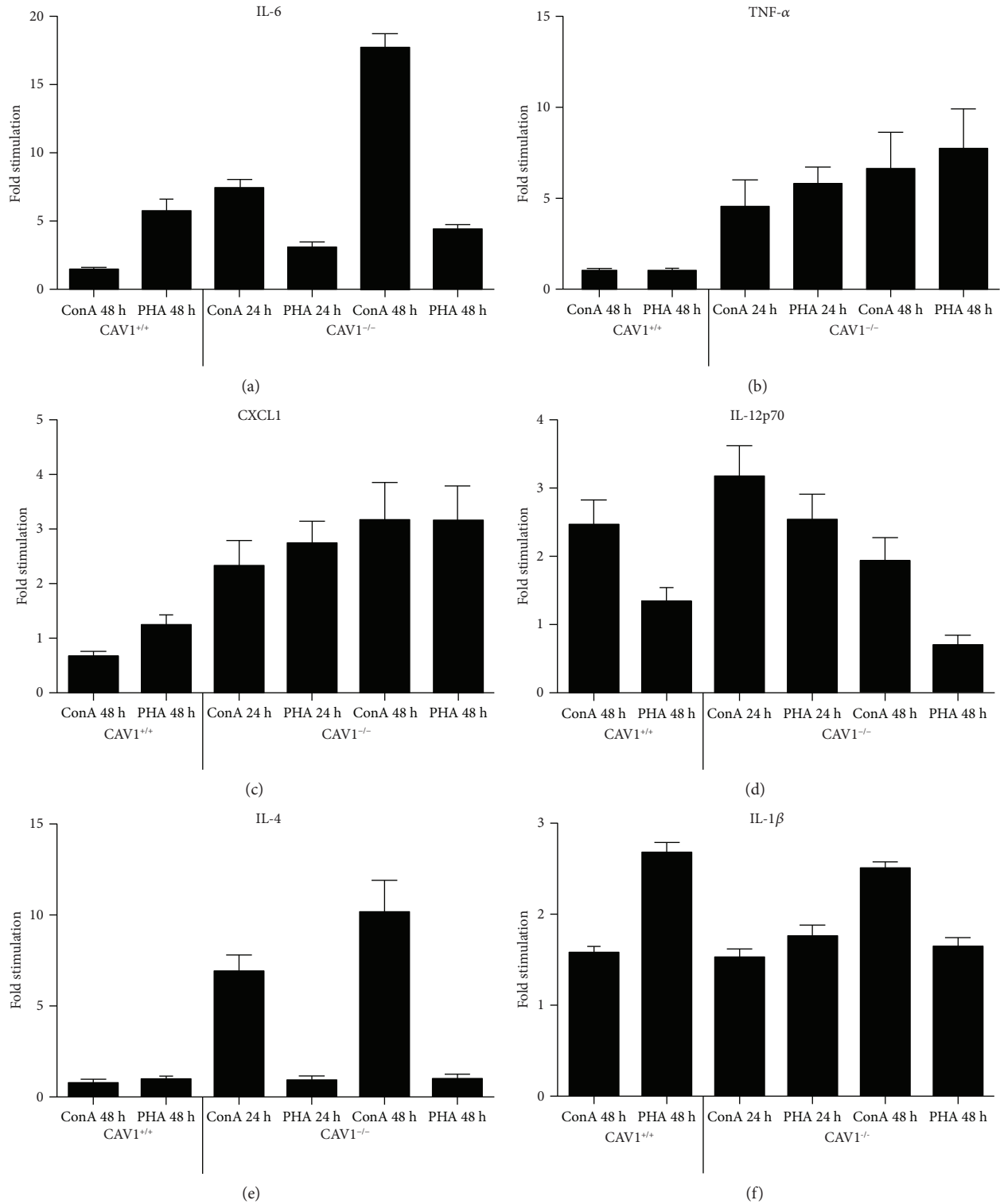
(a)

(b)

(c)

(d)

(e)

(f)

FIGURE 3: Cytokine production by lymphocytes upon nonspecific stimulation. Cytokine levels in the cell culture media of whole blood cells, treated with nonspecific lymphocyte stimuli at different time points after stimulation, as assessed by the xMAP multiplex assay: IL-6 (a), TNF-α (b), CXCL1 (c), IL-12p70 (d), IL-4 (e), and IL-1β (f). The data represent the fold modification of the cytokines from the CAV1$^{-/-}$-derived cells versus the controls.

compensate for the increase in the proinflammatory cytokines (IL-6 and TNF-α), but further investigation would be needed to validate such a mechanism.

The significant increase in IL-6 was confirmed by the Proteome Profiler, along with that in TNF-α and IL-12p70 (Figure 2). However, not all of the tested cytokines showed

a modification of their circulation levels, compared with those in the control. As CAV1 has been previously described as an inhibitor of cell signaling, a loss of function can be correlated with the activation of different signaling pathways that result in subsequent cytokine production [51]. The significant increase in IL-6 reported in our study correlates with the activation of the JAK2/STAT3 signaling pathway reported by Yuan et al. in the lung endothelium of CAV1-KO mice [62], which was involved in *IL-6* gene transcription.

4.2. CAV1$^{-/-}$ Lymphocytes Produce Enhanced Levels of Cytokines upon Stimulation. Endothelial cells and lymphocytes are common cellular sources of cytokines, and it has been demonstrated that endothelial inflammation is suppressed by CAV1 under physiological conditions [63]. Although uncontrolled inflammatory responses have been reported previously in relation to the loss of CAV1 [64], most of these studies have addressed the involvement of the lung endothelium [20, 21]. Owing to the abundance of caveolae in endothelial cells, these cells were the main target of CAV1 inflammation studies [10, 21, 65, 66]. To complement those studies, we addressed the contribution of other cells, namely, circulating lymphocytes, to cytokine production. Lymphocytes were initially considered negative controls for CAV1 expression, as they do not form caveolae, unless transfected with CAV1 [67]. Caveolin-1 has been detected and reported in leukemic cells [68, 69], possibly in conjunction with its involvement in cancer, and may reflect tumorigenic changes. We tested the response of CAV1-KO lymphocytes to nonspecific stimulation, which was quantified by the cytokine output in cell culture, as a possible tool to study low-grade inflammation. Our results showed that CAV1-KO lymphocytes are responsible for the production of various types of pro- and anti-inflammatory cytokines, depending on the type of stimulation and exposure time (Figures 3(a) and 3(f)). These results are supported by a number of studies reporting the role of CAV1 in primary T cells [48] and splenic B cells stimulated with LPS [27], as well as a recent report underlining the involvement of CAV1 in the regulation of B cell tolerance [47].

5. Conclusions

A CAV1-KO mouse model has been intensely used as a tool to study endothelial dysfunction, as well as tumor biology, owing to the increased susceptibility of these mice to cancer [70]. We hypothesized that CAV1 loss could also be involved in inflammation, which is a common feature of many pathologies, from cardiovascular diseases to tumor development. We demonstrated the existence of a low-grade systemic inflammatory milieu, characterized by moderately increased plasma levels of IL-6, TNF-α, and IL12-70p. Circulating lymphocytes of the CAV1$^{-/-}$ mice were overresponsive to stimuli, indicating that these cells may contribute to the maintenance of this low-grade systemic inflammatory environment. Lymphocytes could also prove to be a useful tool to assess anticancer therapies that target inflammation. Our findings showed that CAV1-KO mice can also be used as an *in vivo* model for studying inflammation and could serve

in the assessment of the anti-inflammatory effect of potential novel therapies. Given the strong association between inflammation and cancer [71–73], CAV1-KO mice may be useful for studies focusing on the intricate connections between inflammation and cancer. Finally, it could be added that CAV1, besides being a tumor suppressor, can also act as an inflammation suppressor that can be considered in the studies on CAV1-null tumors.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Disclosure

Conflicts of Interest

The authors declare that there are no conflicts of interests regarding the publication of this paper.

Authors' Contributions

Elena Codrici, Lucian Albulescu, Ionela Daniela Popescu, and Simona Mihai contributed equally to this work.

Acknowledgments

References

[1] D. Calcada, D. Vianello, E. Giampieri et al., "The role of low-grade inflammation and metabolic flexibility in aging and nutritional modulation thereof: a systems biology approach," *Mechanisms of ageing and development*, vol. 136-137, pp. 138–147, 2014.

[2] A. Bektas, S. H. Schurman, R. Sen, and L. Ferrucci, "Aging, inflammation and the environment," *Experimental gerontology*, vol. 105, pp. 10–18, 2018.

[3] R. H. Straub and C. Schradin, "Chronic inflammatory systemic diseases – an evolutionary trade-off between acutely beneficial but chronically harmful programs," *Evolution, Medicine, & Public Health*, vol. 2016, no. 1, pp. 37–51, 2016.

[4] T. Yalta and K. Yalta, "Systemic inflammation and arrhythmogenesis: a review of mechanistic and clinical perspectives," *Angiology*, vol. 69, no. 4, pp. 288–296, 2018.

[5] S. Radenovic, G. Loncar, A. Busjahn et al., "Systemic inflammation and functional capacity in elderly heart failure patients," *Clinical research in cardiology*, vol. 107, no. 4, pp. 362–367, 2018.

[6] A. Vallee and Y. Lecarpentier, "Crosstalk between peroxisome proliferator-activated receptor gamma and the canonical

WNT/β-catenin pathway in chronic inflammation and oxidative stress during carcinogenesis," *Frontiers in immunology*, vol. 9, p. 745, 2018.

[7] Y. Liu and X. Cao, "Characteristics and significance of the pre-metastatic niche," *Cancer Cell*, vol. 30, no. 5, pp. 668–681, 2016.

[8] H. Braumuller, T. Wieder, E. Brenner et al., "T-helper-1-cell cytokines drive cancer into senescence," *Nature*, vol. 494, no. 7437, pp. 361–365, 2013.

[9] X. Yan, S. C. Jiao, G. Q. Zhang, Y. Guan, and J. L. Wang, "Tumor-associated immune factors are associated with recurrence and metastasis in non-small cell lung cancer," *Cancer gene therapy*, vol. 24, no. 2, pp. 57–63, 2017.

[10] E. E. Powter, P. R. Coleman, M. H. Tran et al., "Caveolae control the anti-inflammatory phenotype of senescent endothelial cells," *Aging Cell*, vol. 14, no. 1, pp. 102–111, 2015.

[11] D. M. Yu, S. H. Jung, H. T. An et al., "Caveolin-1 deficiency induces premature senescence with mitochondrial dysfunction," *Aging Cell*, vol. 16, no. 4, pp. 773–784, 2017.

[12] S. P. Basourakos, J. W. Davis, B. F. Chapin et al., "Baseline and longitudinal plasma caveolin-1 level as a biomarker in active surveillance for early-stage prostate cancer," *BJU international*, vol. 121, no. 1, pp. 69–76, 2018.

[13] M. Seker, D. Aydin, A. Bilici et al., "Correlation of caveolin-1 expression with prognosis in patients with gastric cancer after gastrectomy," *Oncology research and treatment*, vol. 40, no. 4, pp. 185–190, 2017.

[14] K. Quann, D. M. Gonzales, I. Mercier et al., "Caveolin-1 is a negative regulator of tumor growth in glioblastoma and modulates chemosensitivity to temozolomide," *Cell Cycle*, vol. 12, no. 10, pp. 1510–1520, 2013.

[15] G. Ayala, M. Morello, A. Frolov et al., "Loss of caveolin-1 in prostate cancer stroma correlates with reduced relapse-free survival and is functionally relevant to tumour progression," *The Journal of pathology*, vol. 231, no. 1, pp. 77–87, 2013.

[16] A. K. Witkiewicz, J. Kline, M. Queenan et al., "Molecular profiling of a lethal tumor microenvironment, as defined by stromal caveolin-1 status in breast cancers," *Cell Cycle*, vol. 10, no. 11, pp. 1794–1809, 2011.

[17] X. Zhao, Y. He, J. Gao et al., "Caveolin-1 expression level in cancer associated fibroblasts predicts outcome in gastric cancer," *PLoS One*, vol. 8, no. 3, article e59102, 2013.

[18] C. J. G. de Almeida, "Caveolin-1 and caveolin-2 can be antagonistic partners in inflammation and beyond," *Frontiers in immunology*, vol. 8, p. 1530, 2017.

[19] L. Qin, N. Zhu, B. X. Ao et al., "Caveolae and caveolin-1 integrate reverse cholesterol transport and inflammation in atherosclerosis," *International journal of molecular sciences*, vol. 17, no. 3, p. 429, 2016.

[20] S. Garrean, X. P. Gao, V. Brovkovych et al., "Caveolin-1 regulates NF-κB activation and lung inflammatory response to sepsis induced by lipopolysaccharide," *The Journal of Immunology*, vol. 177, no. 7, pp. 4853–4860, 2006.

[21] H. Jiao, Y. Zhang, Z. Yan et al., "Caveolin-1 Tyr[14] phosphorylation induces interaction with TLR4 in endothelial cells and mediates MyD88-dependent signaling and sepsis-induced lung inflammation," *The Journal of immunology*, vol. 191, no. 12, pp. 6191–6199, 2013.

[22] A. Kataki, I. Karagiannidis, N. Memos et al., "Host's endogenous caveolin-1 expression is downregulated in the lung during sepsis to promote cytoprotection," *Shock*, vol. 50, no. 2, pp. 199–208, 2017.

[23] J. Millan, L. Hewlett, M. Glyn, D. Toomre, P. Clark, and A. J. Ridley, "Lymphocyte transcellular migration occurs through recruitment of endothelial ICAM-1 to caveola- and F-actin-rich domains," *Nature cell biology*, vol. 8, no. 2, pp. 113–123, 2006.

[24] D. Engel, L. Beckers, E. Wijnands et al., "Caveolin-1 deficiency decreases atherosclerosis by hampering leukocyte influx into the arterial wall and generating a regulatory T-cell response," *FASEB journal*, vol. 25, no. 11, pp. 3838–3848, 2011.

[25] C. T. Damsgaard, L. Lauritzen, P. C. Calder, T. M. R. Kjær, and H. Frøkiær, "Whole-blood culture is a valid low-cost method to measure monocytic cytokines - a comparison of cytokine production in cultures of human whole-blood, mononuclear cells and monocytes," *Journal of immunological methods*, vol. 340, no. 2, pp. 95–101, 2009.

[26] M. I. Lin, J. Yu, T. Murata, and W. C. Sessa, "Caveolin-1-deficient mice have increased tumor microvascular permeability, angiogenesis, and growth," *Cancer research*, vol. 67, no. 6, pp. 2849–2856, 2007.

[27] F. A. Medina, C. J. de Almeida, E. Dew et al., "Caveolin-1-deficient mice show defects in innate immunity and inflammatory immune response during *Salmonella enterica* serovar Typhimurium infection," *Infection and immunity*, vol. 74, no. 12, pp. 6665–6674, 2006.

[28] I. Langezaal, S. Hoffmann, T. Hartung, and S. Coecke, "Evaluation and prevalidation of an immunotoxicity test based on human whole-blood cytokine release," *Alternatives to laboratory animals: ATLA*, vol. 30, no. 6, pp. 581–595, 2002.

[29] C. P. Tanase, "Caveolin-1: a marker for pancreatic cancer diagnosis," *Expert review of molecular diagnostics*, vol. 8, no. 4, pp. 395–404, 2008.

[30] P. E. Kobayashi, C. E. Fonseca-Alves, L. G. Rivera-Calderón et al., "Deregulation of E-cadherin, β-catenin, APC and caveolin-1 expression occurs in canine prostate cancer and metastatic processes," *Research in veterinary science*, vol. 118, pp. 254–261, 2018.

[31] P. Hammarsten, T. Dahl Scherdin, C. Hägglöf et al., "High caveolin-1 expression in tumor stroma is associated with a favourable outcome in prostate cancer patients managed by watchful waiting," *PLoS One*, vol. 11, no. 10, article e0164016, 2016.

[32] J. Yeong, A. A. Thike, M. Ikeda et al., "Caveolin-1 expression as a prognostic marker in triple negative breast cancers of Asian women," *Journal of clinical pathology*, vol. 71, no. 2, pp. 161–167, 2018.

[33] B. K. Ryu, M. G. Lee, N. H. Kim et al., "Bidirectional alteration of Cav-1 expression is associated with mitogenic conversion of its function in gastric tumor progression," *BMC Cancer*, vol. 17, no. 1, p. 766, 2017.

[34] W. Liu, N. C. Yin, H. Liu, and K. J. Nan, "Cav-1 promote lung cancer cell proliferation and invasion through lncRNA HOTAIR," *Gene*, vol. 641, pp. 335–340, 2018.

[35] F. Meng, S. Saxena, Y. Liu et al., "The phospho-caveolin-1 scaffolding domain dampens force fluctuations in focal adhesions and promotes cancer cell migration," *Molecular biology of the cell*, vol. 28, no. 16, pp. 2190–2201, 2017.

[36] C. P. Tanase, S. Dima, M. Mihai et al., "Caveolin-1 overexpression correlates with tumour progression markers in pancreatic ductal adenocarcinoma," *Journal of molecular histology*, vol. 40, no. 1, pp. 23–29, 2009.

[37] W. Yang, T. Xu, P. Qiu, and G. Xu, "Caveolin-1 promotes pituitary adenoma cells migration and invasion by regulating the interaction between EGR1 and KLF5," *Experimental cell research*, vol. 367, no. 1, pp. 7–14, 2018.

[38] C. Tanase, R. Albulescu, E. Codrici et al., "Decreased expression of APAF-1 and increased expression of cathepsin B in invasive pituitary adenoma," *OncoTargets and therapy*, vol. 8, pp. 81–90, 2015.

[39] S. H. Chang, D. Feng, J. A. Nagy, T. E. Sciuto, A. M. Dvorak, and H. F. Dvorak, "Vascular permeability and pathological angiogenesis in caveolin-1-null mice," *The American journal of pathology*, vol. 175, no. 4, pp. 1768–1776, 2009.

[40] W. A. Shihata, D. L. Michell, K. L. Andrews, and J. P. F. Chin-Dusting, "Caveolae: a role in endothelial inflammation and mechanotransduction?," *Frontiers in Physiology*, vol. 7, p. 628, 2016.

[41] P. X. Zhang, J. Cheng, S. Zou et al., "Pharmacological modulation of the AKT/microRNA-199a-5p/CAV1 pathway ameliorates cystic fibrosis lung hyper-inflammation," *Nature Communications*, vol. 6, no. 1, p. 6221, 2015.

[42] S. G. Royce and C. J. Le Saux, "Role of caveolin-1 in asthma and chronic inflammatory respiratory diseases," *Expert review of respiratory medicine*, vol. 8, no. 3, pp. 339–347, 2014.

[43] J. H. Chidlow Jr., J. J. Greer, C. Anthoni et al., "Endothelial caveolin-1 regulates pathologic angiogenesis in a mouse model of colitis," *Gastroenterology*, vol. 136, no. 2, pp. 575–584.e2, 2009.

[44] L. Xie, X. Xue, M. Taylor et al., "Hypoxia-inducible factor/ MAZ-dependent induction of caveolin-1 regulates colon permeability through suppression of occludin, leading to hypoxia-induced inflammation," *Molecular and cellular biology*, vol. 34, no. 16, pp. 3013–3023, 2014.

[45] X. Li, X. Gu, T. M. Boyce et al., "Caveolin-1 increases proinflammatory chemoattractants and blood-retinal barrier breakdown but decreases leukocyte recruitment in inflammation," *Investigative ophthalmology & visual science*, vol. 55, no. 10, pp. 6224–6234, 2014.

[46] F. A. Medina, A. W. Cohen, C. J. de Almeida et al., "Immune dysfunction in caveolin-1 null mice following infection with Trypanosoma cruzi (Tulahuen strain)," *Microbes and infection*, vol. 9, no. 3, pp. 325–333, 2007.

[47] S. Minguet, K. Klasener, A. M. Schaffer et al., "Caveolin-1-dependent nanoscale organization of the BCR regulates B cell tolerance," *Nature Immunology*, vol. 18, no. 10, pp. 1150–1159, 2017.

[48] T. Tomassian, L. A. Humphries, S. D. Liu, O. Silva, D. G. Brooks, and M. C. Miceli, "Caveolin-1 orchestrates TCR synaptic polarity, signal specificity, and function in CD8 T cells," *Journal of immunology*, vol. 187, no. 6, pp. 2993–3002, 2011.

[49] T. Shiroto, N. Romero, T. Sugiyama et al., "Caveolin-1 is a critical determinant of autophagy, metabolic switching, and oxidative stress in vascular endothelium," *PLoS One*, vol. 9, no. 2, article e87871, 2014.

[50] S. Wang, N. Wang, Y. Zheng, J. Zhang, F. Zhang, and Z. Wang, "Caveolin-1: an oxidative stress-related target for cancer prevention," *Oxidative medicine and cellular longevity*, vol. 2017, Article ID 7454031, 20 pages, 2017.

[51] S. Pavlides, A. Tsirigos, I. Vera et al., "Loss of stromal caveolin-1 leads to oxidative stress, mimics hypoxia and drives inflammation in the tumor microenvironment, conferring the "reverse Warburg effect": a transcriptional informatics analysis with validation," *Cell Cycle*, vol. 9, no. 11, pp. 2201–2219, 2010.

[52] U. E. Martinez-Outschoorn, D. Whitaker-Menezes, Z. Lin et al., "Cytokine production and inflammation drive autophagy in the tumor microenvironment: role of stromal caveolin-1 as a key regulator," *Cell Cycle*, vol. 10, no. 11, pp. 1784–1793, 2011.

[53] D. Volonte, Z. Liu, P. M. Musille et al., "Inhibition of nuclear factor-erythroid 2-related factor (Nrf2) by caveolin-1 promotes stress-induced premature senescence," *Molecular biology of the cell*, vol. 24, no. 12, pp. 1852–1862, 2013.

[54] A. Cuadrado, G. Manda, A. Hassan et al., "Transcription factor NRF2 as a therapeutic target for chronic diseases: a systems medicine approach," *Pharmacological Reviews*, vol. 70, no. 2, pp. 348–383, 2018.

[55] W. Li, H. Liu, J. S. Zhou et al., "Caveolin-1 inhibits expression of antioxidant enzymes through direct interaction with nuclear erythroid 2 p45-related factor-2 (Nrf2)," *The Journal of biological chemistry*, vol. 287, no. 25, pp. 20922–20930, 2012.

[56] J. Mauer, J. L. Denson, and J. C. Bruning, "Versatile functions for IL-6 in metabolism and cancer," *Trends in immunology*, vol. 36, no. 2, pp. 92–101, 2015.

[57] Y. A. Daisuke Kamimura, T. Hirano, H. Ogura, and M. Murakami, "IL-6 and inflammatory diseases," in *Cytokine Frontiers, Regulation of Immune Responses in Health and Disease*, T. Yoshimoto and T. Yoshimoto, Eds., Springer, Tokyo, 2014.

[58] C. Y. Lee, T. Y. Lai, M. K. Tsai et al., "The ubiquitin ligase ZNRF1 promotes caveolin-1 ubiquitination and degradation to modulate inflammation," *Nature Communications*, vol. 8, p. 15502, 2017.

[59] Y. Yamaguchi, Y. Watanabe, T. Watanabe, N. Komitsu, and M. Aihara, "Decreased expression of caveolin-1 contributes to the pathogenesis of psoriasiform dermatitis in mice," *The Journal of investigative dermatology*, vol. 135, no. 11, pp. 2764–2774, 2015.

[60] T. Zhu, Q. Meng, J. Ji, L. Zhang, and X. Lou, "TLR4 and caveolin-1 in monocytes are associated with inflammatory conditions in diabetic neuropathy," *Clinical and translational science*, vol. 10, no. 3, pp. 178–184, 2017.

[61] C. R. Weiss, Q. Guan, Y. Ma et al., "The potential protective role of caveolin-1 in intestinal inflammation in TNBS-induced murine colitis," *PLoS One*, vol. 10, no. 3, article e0119004, 2015.

[62] K. Yuan, C. Huang, J. Fox et al., "Elevated inflammatory response in caveolin-1-deficient mice with *Pseudomonas aeruginosa* infection is mediated by STAT3 protein and nuclear factor κB (NF-κB)," *The Journal of biological chemistry*, vol. 286, no. 24, pp. 21814–21825, 2011.

[63] M. Bucci, J. P. Gratton, R. D. Rudic et al., "In vivo delivery of the caveolin-1 scaffolding domain inhibits nitric oxide synthesis and reduces inflammation," *Nature medicine*, vol. 6, no. 12, pp. 1362–1367, 2000.

[64] H. Feng, L. Guo, Z. Song et al., "Caveolin-1 protects against sepsis by modulating inflammatory response, alleviating bacterial burden, and suppressing thymocyte apoptosis," *The Journal of biological chemistry*, vol. 285, no. 33, pp. 25154–25160, 2010.

[65] S. M. Grenon, M. Jeanne, J. Aguado-Zuniga, M. S. Conte, and M. Hughes-Fulford, "Effects of gravitational mechanical unloading in endothelial cells: association between caveolins, inflammation and adhesion molecules," *Scientific Reports*, vol. 3, no. 1, p. 1494, 2013.

[66] M. K. Mirza, J. Yuan, X. P. Gao et al., "Caveolin-1 deficiency dampens Toll-like receptor 4 signaling through eNOS activation," *The American journal of pathology*, vol. 176, no. 5, pp. 2344–2351, 2010.

[67] J. Vallejo and C. D. Hardin, "Expression of caveolin-1 in lymphocytes induces caveolae formation and recruitment of phosphofructokinase to the plasma membrane," *FASEB journal*, vol. 19, no. 6, pp. 586-587, 2005.

[68] M. Hatanaka, T. Maeda, T. Ikemoto, H. Mori, T. Seya, and A. Shimizu, "Expression of caveolin-1 in human T cell leukemia cell lines," *Biochemical and biophysical research communications*, vol. 253, no. 2, pp. 382–387, 1998.

[69] Y. Tsuji, T. Nakagawa, M. Hatanaka et al., "Quantification of caveolin isoforms using quantitative real-time RT-PCR, and analysis of promoter CpG methylation of caveolin-1alpha in human T cell leukemia cell lines," *International journal of molecular medicine*, vol. 18, no. 3, pp. 489–495, 2006.

[70] S. Le Lay and T. V. Kurzchalia, "Getting rid of caveolins: phenotypes of caveolin-deficient animals," *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, vol. 1746, no. 3, pp. 322–333, 2005.

[71] L. M. Coussens and Z. Werb, "Inflammation and cancer," *Nature*, vol. 420, no. 6917, pp. 860–867, 2002.

[72] J. G. Fox and T. C. Wang, "Inflammation, atrophy, and gastric cancer," *Journal of Clinical Investigation*, vol. 117, no. 1, pp. 60–69, 2007.

[73] M. A. Dobrovolskaia and S. V. Kozlov, "Inflammation and cancer: when NF-κB amalgamates the perilous partnership," *Current cancer drug targets*, vol. 5, no. 5, pp. 325–344, 2005.