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Criminal Regulatory Approaches to Deepfake-Related Offenses: Focusing on the Crime of Fraud

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Abstract

Coronary artery disease (CAD), which is driven primarily by atherosclerosis, represents a major global health burden. This review explores the dynamic evolution of the CAD immune microenvironment through single-cell RNA sequencing (scRNA-seq), revealing cellular heterogeneity and interactions. On the basis of the American Heart Association's histological classification of atherosclerotic lesions, we systematically summarize discoveries supported by scRNA-seq across disease stages (types I-V) from early monocyte recruitment and lipid-resistant macrophage subpopulations (e.g., CD52-hi macrophages) in initial lesions to intensified inflammation involving T-cell activation, NK cell differentiation, and macrophage polarization in fatty streaks and intermediate lesions and late-stage fibrosis and T-cell clonal expansion leading to plaque instability. Key findings highlight immune imbalance exacerbated by comorbidities such as diabetes and systemic lupus erythematosus (SLE), with potential biomarkers (e.g., JUN) and therapeutic targets (e.g., the CCL4–CCR5 axis and SPP1+ macrophages). Methodological limitations, such as the lack of spatial information and challenges in inferring causal relationships, are discussed. Future prospects, such as spatial transcriptomics integration, multiomics approaches, and AI-assisted precision medicine, are also proposed. This review highlights the transformative role of scRNA-seq in advancing CAD pathology toward precision diagnostics and therapies.

Keywords

single-cell RNA sequencing, coronary artery disease, immune microenvironment, atherosclerosis, cellular heterogeneity

1. Introduction

Coronary artery disease (CAD) is one of the leading causes of death worldwide[1, 2]. The global burden of CAD is reflected not only in high mortality rates but also in high morbidity and healthcare costs. Many CAD patients require long-term medication therapy, interventional treatments (such as percutaneous coronary intervention (PCI)), or surgical treatments (such as coronary artery bypass grafting (CABG)) [2, 3]. These treatment methods not only impose financial burdens on patients but also place enormous pressure on healthcare systems. Therefore, reducing the global burden of CAD requires collective efforts worldwide, including raising public health awareness, improving lifestyles, strengthening early screening and diagnosis, and developing more effective treatment methods.

In recent years, a growing body of research has demonstrated that the immune microenvironment plays a crucial role in the occurrence and development of CAD[4-6]. Atherosclerosis, the primary pathological mechanism underlying CAD, is recognized as a chronic inflammatory disease [7]. In its early stages, immune cells such as monocytes and T cells infiltrate the arterial intima [8], where inflammatory factors affect them to activate vascular endothelial cells, promoting lipid deposition and oxidation that lead to plaque formation. Multiple immune cell subsets, including macrophages, T cells, B cells, and natural killer cells, exist in CAD patients [8]. These immune cells exacerbate vascular inflammatory responses and promote the formation and rupture of atherosclerotic plaques by releasing inflammatory factors and cytotoxic molecules.

During the development of coronary atherosclerotic disease (CAD), the composition and function of resident immune cells play crucial roles in plaque formation, stabilization, and rupture. However, traditional bulk transcriptomics and histology have difficulty differentiating cell subpopulations and their transitions at the single-cell level. Single-cell RNA sequencing (scRNA-seq) provides individual-cell resolution, making it possible to analyze the heterogeneity and functional differentiation of immune cells and their cellular interactions [9].

On the basis of the AHA's classification of atherosclerosis progression and further research [10-14], this paper systematically reviews the major biological discoveries, methodological limitations of scRNA-seq in CAD research, and technologies and research methods that are expected to solve these problems, aiming to identify verifiable and translatable directions for future research.

2. Single-Cell RNA Sequencing Technologies

To understand how single-cell RNA sequencing (scRNA-seq) shapes the current understanding of the immune microenvironment of CAD, this section outlines the key sample preparation, sequencing platforms, and commonly used analysis procedures and focuses on the application of this technology in CAD research. The first two steps of scRNA-seq involve separating individual cells and constructing sequencing libraries. The methods for cell separation include mechanical separation, enzymatic separation, and flow cytometry (FACS) or microfluidic sorting [15, 16]. The methods for library construction mainly include the Smart-seq series, the 10x Genomics platform, and Drop-seq, among which each method has its own characteristics and is suitable for different experimental needs [17-19]. Luecken et al. provided a tutorial of workflows for single-cell RNA-seq analysis [20], including preprocessing and downstream analysis. Raw data are processed to obtain count matrices, and quality control, normalization, dimensionality reduction, visualization and other steps are usually performed to provide a reliable, low-dimensional, and comparable gene expression matrix. After preprocessing, researchers can conduct clustering, trajectory inference, differential expression and other analytic steps. With various protocols and analysis tools, scRNA-seq reveals great advantages in the inference of cell types, functional states, and interaction networks in CAD. Moreover, this technology can also be integrated with multiomics (e.g., CITE-seq, scATAC-seq), providing a more comprehensive perspective.

Single-cell RNA sequencing (scRNA-seq) has shifted from static bulk RNA analysis, which masks cellular heterogeneity by averaging signals, to single-cell resolution, revealing dynamic immune trajectories and subpopulations in the coronary artery disease (CAD) immune microenvironment. These findings provide detailed insights into atherosclerosis progression across pathological stages. Overall, tools based on scRNA-seq provide a robust framework for researching the immune dynamics of CAD, as detailed in the following sections.

3. Main Body

3.1 Type I: Initial Lesion

In this stage, the immune environment is characterized mainly by mild inflammation, with the accumulation of isolated macrophages. Its major activities include endothelial injury, initial recruitment of monocytes and preliminary formation of foam cells.

In the coronary vascular wall, scRNAA-seq, along with snATAC-seq, has revealed 14 different cell types, including smooth muscle smooth muscle cells (SMCs), endothelial cells, fibroblasts, macrophages, T cells and other types of cells [21]. Analysis at the mononuclear level has greatly improved the ability to distinguish rare

cell types. In the development of arterial atherosclerosis, an increased proportion of immune cells indicates their participation in the early stage of lesion development[21]. To understand how these cells contribute to disease, further research has investigated the gene regulatory mechanisms affected by CAD risk variants. The study revealed that the specific chromatin accessible region of these cell types may be affected by CAD risk genes[21]. In support of this result, the enrichment of crucial TFs (e.g., TCF21, PRDM16, and TBX2) [21] in several specific cell types (e.g., SMCs and fibroblasts) suggests their elusive roles in the early initiation or maintenance of cellular phenotype transformation in disease.

Kirichenko et al. revealed the increased proinflammatory activation of monocytes in diabetes mellitus and suggested that it is the cause of accelerated atherogenesis. These findings suggest that the development of atherosclerosis may be affected by comorbidities [22]. Indeed, monocyte recruitment is one of the main pathological manifestations in this stage, as explored in another study investigating monocyte heterogeneity in the initiation of artery atherosclerosis [23]. Using advanced single-cell technologies such as CITE-seq and single-cell RNA sequencing, this study identified novel monocyte subpopulations—such as MHCIIhi, IFN-responsive, and monocyte–platelet aggregates—and their associations with CAD risk factors such as hyperlipidemia, smoking, and race. These subpopulations exhibit distinct transcriptional and phenotypic profiles, with specific alterations linked to CAD risk states, suggesting their potential as biomarkers or therapeutic targets for CAD.

The preliminary formation of foam cells is another pathological manifestation of the initial lesion. After being recruited under the vascular endothelium, monocytes, crucial infiltrating cells, differentiate into macrophages [24] and accumulate, providing the basis for foam cell formation. Using scRNA-seq and multitomic approaches, Jiang et al. [25] discovered that CD52-hi macrophages, which are enriched in the genetic heritability of CAD, accumulate significantly fewer lipoproteins with activated lipid metabolism pathways. It has a strong ability to remove and process lipoproteins in the early stage of arterial atherosclerosis. The mechanisms may involve the upregulation of certain lipid transport proteins and metabolic enzymes or the downregulation of pathways leading to lipid accumulation to resist lipid overload. This ability can be helpful in slowing down or prohibiting the formation and development of plaques.

In the initial lesion stage, scRNA-seq and snATAC-seq identify diverse cell types within the microenvironment and regulatory impacts from CAD risk genes. Single-cell RNA sequencing revealed the heterogeneity of monocytes and the lipid resistance mechanism of CD52hi macrophages, laying the foundation for atherosclerosis. Although the inflammation was mild, risk factors such as diabetes exacerbated monocyte activation, suggesting that prevention strategies should target initial recruitment. Discoveries at this stage provided molecular insights into the transition to fatty streaks.

3.2 Type II Fatty Streaks

In this stage, along with intimal thickening, multilayered foam cells, and lipid deposition, the inflammatory microenvironment intensifies, with T cells and B cells beginning to participate, and proinflammatory factors increase.

Dendritic cells and macrophages play critical roles in promoting inflammation in the development of arterial atherosclerosis. Dendritic cells present antigens to T lymphocytes to bridge innate and adaptive immune responses. Macrophages exhibit polarization during atherosclerosis, with M1-type macrophages and M2-type macrophages showing opposite responses to inflammation [26]. Although M1/M2 polarization provides a useful framework for understanding the function of macrophages, in the complex microenvironment of atherosclerosis, the behavior of macrophages is far from simple binary opposition. They may exhibit a continuous spectrum or a mixed phenotype. Its function may be highly dependent on dynamic changes in local signals (such as oxidized lipids, cytokines, and hypoxia). In addition, in the same study, T cells and macrophages were linked to the release of DAMPs and PRR activation in artery atherosclerosis, but the causal relationship is still unknown. Research by Hortsmann revealed enrichment of proliferation-associated pathways in several subtypes of NK cells and T cells and pathways linked with immune cell activation, signaling through immune receptors, and differentiation in T and B lymphocytes [27]. The detection of restricted T-cell receptor sequences in multiple adaptive immune cell subtypes from CAD patients points to enhanced cellular clonality and antigen specificity. These findings indicate the specific differentiation of T

cells in CAD [27]. These results validated the immunological expansion in the fatty streak stage at the molecular level.

The research team of Li selected a crucial C1 RACK1+ NK cell subgroup, which has differential potential in the formation of coronary plaques according to CytoTrace [28]. Although NK cells do not participate in lipid deposition directly, the C1 RACK1+ NK cell subset may indirectly regulate the lipid uptake ability of macrophages or smooth muscle cells by secreting cytokines or chemokines, thus altering the speed of lipid deposition. Research in this area could provide a key mechanistic basis for understanding the inflammatory cascade of atherosclerosis and for developing antiplaque therapeutic strategies targeting NK cell function.

In this stage, more macrophages generally transform into foam cells by engulfing lipids, revealing the core characteristics of plaque formation. Research has also revealed that the activation and differentiation of T cells, especially CD4+ T cells, regulate inflammatory reactions. The number of recruited cells in the Type I stage further increases, and these cells differentiate [24]. For example, SMCs display significant phenotypic plasticity in the development of artery atherosclerosis and can differentiate into fibromyocytes, which are associated with specific TFs (e.g., AP-1, TCF21) and genes (TNFRSF11B, FN1) [21]. In the same study, some TFs (e.g., PU.1/SPIB, IRF) and CAD risk gene loci (e.g., GWAS SNPs) were significantly enriched in macrophages during the progression of artery atherosclerosis [21]. These regulatory relationships between cells and their regulators are critical for understanding pathological mechanisms and immune reactions in artery atherosclerosis.

Another study [29]explored the characteristics of peripheral circulating immune cells and identified JUN as a highly active regulon within monocyte clusters via scRNA-seq. Further analysis revealed that JUN expression was significantly upregulated in the CAD group. On the basis of these findings, JUN may serve as a potential diagnostic biomarker and predictor for CAD, suggesting that the JUN signaling pathway is a possible therapeutic target. This study reveals the significant activity of the JUN regulon in single cells of CAD patients through advanced single-cell analysis technology, which is a very promising finding. However, correlation analysis alone is not sufficient to establish causality. Is the upregulation of JUN a driving factor for the initiation of CAD or a secondary reaction in the atherosclerotic inflammatory environment? Moreover, does the sample cohort of the study cover patients of different stages and subtypes of CAD to ensure the general applicability of JUN as a biomarker? These issues require further large-scale and more refined stratified research to be confirmed.

The analysis of scRNA-seq data during the fatty streak stage revealed an increase in the inflammatory response, including the differentiation of natural killer (NK) cells, macrophage polarization, and an increase in biomarkers such as JUN. These results emphasize how immune cells evolve from initial accumulation to more specialized functions driven by local signaling. This process contributes to the development of more complex intermediate plaques.

3.3 Type III: Intermediate

This stage focuses on the formation of extracellular lipid pools and the complexity of plaques, characterized by an increase in cell interactions within the immune microenvironment, such as the migration of macrophages and smooth muscle cells.

During this pathological period, the complexity of plaques is affected by various factors. Single-cell RNA sequencing (scRNA-seq) and a series of related technologies have revealed high expression of CCL4 in T cells, monocytes and macrophages and increased expression of its receptor CCR5 in atherosclerotic plaques compared with controls [30]. These results suggest that blocking the CCL4–CCR5 interaction is a promising therapeutic target for CAD. However, the effect of blocking the interaction between the CCL4 ligand and its receptor on immune responses in people with CAD still needs to be investigated.

The effect of comorbidities on plaques deserves more attention. Unlike the role of the cytokine IL-1 in the pathogenesis of coronary artery disease (CAD) observed in the general population, the results of this transcriptomic study indicate that type I interferon signaling plays a crucial role in cardiovascular inflammation [31]. When CAD is associated with different diseases, the main pathological factors may change, and the study of these changes has important clinical significance. Similarly, another study on circadian rhythm preliminarily revealed a strong correlation between elevated CRD scores, increased immunoinflammatory activation, and

reduced fibrosis [32]. This association suggests a potential mechanism that could drive the unstable transformation of atherosclerotic plaques.

In addition, the complexity of plaques could be associated with the polarization of cells, such as type II macrophages [26]. For example, a study investigating specific aortic valve diseases via paired scRNA-seq revealed both pro- and anti-inflammatory roles of T cells, and if similar dual-role cells can be found in atherosclerosis, it will help researchers understand the transition in plaque stability and be beneficial for timely and effective treatment.

In this stage, single-cell RNA sequencing (scRNA-seq) revealed an increase in cellular interactions, such as high expression of the CCL4–CCR5 axis in T cells and macrophages and the effects of comorbidities (such as SLE) and circadian rhythm disorders. These findings reveal a critical transition from the peak of inflammation to structural complexity, suggesting potential therapeutic targets.

3.4 Type IV: Advanced Atheroma

This pathological type includes the dominant formation of the lipid core and initial development to the mature covering stage of the fibrous cap, accompanied by the transition from the peak inflammatory response of the immune microenvironment to chronic inflammation. The processes of proliferation and fibrosis around the lipid core accelerate, and regulatory T cells increase their ability to stabilize plaques. This stage is characterized by both the risk of plaque expansion and potential stability evolution features.

SMCs and macrophages play important roles in fibrosis[21], and further studies investigating their associated subgroups and regulatory factors are needed.

Research by Fu et al. [33] revealed that SPP1+ macrophages accumulate in the perivascular adipose tissue (PVAT) of atherosclerotic coronary arteries and exacerbate fibrosis by promoting the migration and proliferation of fibrofatty progenitor cells through OPN-CD44/integrin interactions. The degree of this fibrosis is positively correlated with the severity of coronary artery narrowing, indicating that SPP1+ macrophages in coronary PVAT may play a significant role in the progression of coronary artery atherosclerosis. Single-cell RNA-seq has, for the first time, accurately identified the SPP1+ macrophage subpopulation in atherosclerotic plaques, revealing the specific enrichment of these cells in perivascular adipose tissue (PVAT). Traditional methods have difficulty distinguishing the heterogeneity of macrophage subtypes, but this technology clearly identifies their profibrotic phenotype through gene expression characteristics (such as high expression of SPP1 and MMP9), filling the gap in the understanding of cell functional differentiation in the inflammatory microenvironment.

As a hallmark of CAD, which is explicitly described as primarily resulting from coronary atherosclerosis, ischemic cardiomyopathy (ICM) studies of SMC subgroups can reveal important SMC remodeling at the fibrosis stage of artery atherosclerosis. The critical C6 S100A4+ SMC subpopulation [34], identified through scRNA-seq analysis, interacts with endothelial cells via the PTN-NCL pathway, influencing disease progression. Key transcription factors such as KLF2, FOS, FOSB, and JUNB were identified in this subpopulation, offering potential insights for preventing fibrosis progression. Another study confirmed that previously validated CAD risk sites (such as LMOD1) have specific regulatory specificity in SMCs. Research shows that the regulation of LMOD1 in smooth muscle cells may participate in the mechanism of CHD risk by affecting cell function, particularly aspects such as vascular remodeling and contraction ability [21], which may be related to changes in vascular function in late-stage lesions.

At the gene level, “SMARCA4, translated into protein BRG1, showed significant differences and was found to promote fibroblast proliferation and migration in in vivo experiments.” [35] This discovery holds dual significance for understanding the formation and stability of the ‘fibrous cap’ in atherosclerosis. In the early stages, the moderate proliferation and migration of fibroblasts contribute to the formation of the fibrous cap, which wraps around the lipid core and plays a role in stabilizing the plaque. However, excessive and sustained activation can lead to pathological fibrosis, vascular wall sclerosis, and lumen stenosis. Therefore, the profibrotic effect of SMARCA4/BRG1 is a ‘double-edged sword’, and its specific role depends on the stage of the disease and the degree of activation. These findings suggest that treatment strategies targeting this pathway need to have precise temporal specificity. Compared with traditional bulk sequencing, which can only extract the average signal of cell populations, this highlights the extraordinary value of scRNA-seq technology

in analyzing cellular heterogeneity and identifying key driver genes. Future research should focus on spatial transcriptomics to locate the specific spatial positions of these highly expressed SMARCA4 fibroblasts within the plaque (whether they are in the core of the fibrous cap or near the area of immune cell infiltration), as well as ATAC-seq to directly confirm that BRG1 indeed alters the chromatin accessibility of these cells, which will lay a solid foundation for the development of novel therapeutic strategies for atherosclerosis on the basis of epigenetic regulation.

During this stage, scRNA-seq identified fibrosis processes driven by subtypes such as SPP1+ macrophages and S100A4+ SMCs, revealing the role of genes such as SMARCA4 in cell proliferation. These insights clarify the evolution from chronic inflammation to a stable but progressive pathological period, providing a foundation for understanding late instability and emphasizing the necessity of multiomics integration.

3.5 Type V: Complicated Lesion

In this stage, an acute inflammation outbreak in the immune microenvironment occurs, with platelets and coagulation factors participating. This late type is characterized by plaque rupture, hemorrhage, and thrombosis, leading to acute events.

According to previous studies [24], M1 macrophages (proinflammatory macrophage subtypes) and specific T-cell subtypes (e.g., M1 macrophages). Th1 and Th17 cells) are more active during this period. These cells secrete large amounts of proinflammatory cytokines, exacerbating the inflammation and instability of plaques. In contrast, the functional state of immune suppressor cells such as regulatory T cells (Tregs) may be affected, and their ability to suppress inflammation may weaken, further worsening the proinflammatory process. Unstable plaques are at risk of rupture under the influence of reactions to self-antigens, the cytotoxic pathway, and the exhaustion pathway of CD8+ T cells [36].

HF (heart failure) is an important pathological change that exacerbates myocardial atherosclerosis. Using scRNA-seq, Merten et al. conducted a series of studies [37-39] focused on the activation of T cells and monocytes and the expansion of circulating T cells in the HF. Single-cell RNA sequencing of peripheral immune cells from healthy and HF donors also revealed a significant increase in CD4+ and CD8+ T cells. This study, together with previous studies [36], demonstrated the significant impact of CD8+ T cells in the late stage of atherosclerosis. Upon exposure to the secretome of HFs, endothelial cells increase their ability to adhere to cocultured monocytes and downregulate the level of the checkpoint inhibitor PD-L1 [37, 39], indicating a reduction in the degree of autoimmune reactions by T cells. These findings suggest that endothelial cells are not only targets of inflammatory responses but also active participants in immunity. By downregulating PD-L1, endothelial cells accelerate the recruitment of monocytes and the activation of T cells. In the future, it can be hypothesized that targeting PD-L1 expression in endothelial cells (such as through anti-inflammatory cytokines or epigenetic regulation) can restore immune homeostasis.

scRNA-seq in the complex lesion stage highlights acute inflammation outbreaks, such as the autoimmune response and the clonal expansion of T cells in HFs. These late mechanisms reveal the risk of plaque rupture due to immune imbalance, emphasizing the need for targeted intervention in advanced CAD stages.

4. Discussion and Future Prospects

Using single-cell RNA sequencing technology, researchers have revealed multiple key findings in the immune microenvironment of CAD patients according to the pathological classification framework. In the early stage (types I--II), they explored the lipid resistance of cells such as CD52hi macrophages and the heterogeneity of monocytes [23, 25]. In the middle stage (Type III), T cells are activated by DAMPs, the polarization of macrophages, and the differentiation subtypes of NK cells [26-28]. In the late stage (types IV-V), the field focused on macrophage-driven fibrosis and the expansion of T-cell clones [33, 36-39] and emphasized the dynamic changes across stages (such as from initial inflammation to unstable transformation) and overall patterns (such as immune imbalance amplifying comorbidity effects[22, 24, 31, 32]).

While scRNA-seq has revolutionized CAD research, several limitations exist. (1) Lack of spatial information: Although scRNA-seq captures heterogeneity, it ignores tissue localization, leading to an incomplete understanding of local mechanisms such as PVAT fibrosis [33] or endothelial interactions [34]. (2) Insufficient causal validation: Since scRNA-seq is a technology that relies on complex data analysis to reveal

the characteristics of single-cell transcriptomes, many of its findings (such as JUN upregulation[29] and the CCL4–CCR5 axis [30]) remain at the level of association and cannot distinguish causality, especially with the limitation of a small sample size in generalization. (3) Lack of integration of comorbidities: Although it touches on diabetes and SLE[22, 24, 31], it lacks multiomics validation, resulting in insufficient comprehensiveness of immune changes.

Considering these limitations, several directions for future research should be explored further. (1) Integrating spatial transcriptomics with scRNA-seq can supplement positional information, further analyzing cellular interactions such as those in the cores of plaques. (2) Single-cell multiomics (e.g., scATAC-seq + scRNA-seq) can elucidate gene regulation, such as by verifying the role of TFs (such as TCF21 and JUNB[21, 34]) in heterogeneous subtypes. (3) AI-assisted analysis can be used for early intervention (e.g., through machine learning [21] for personalized prediction of disease trajectories). (4) In clinical translation, targeted immunotherapies for subtypes (such as SPP1+ macrophages [33]and S100A4+ SMCs [34]) or circulating biomarkers (such as JUN[29]) can be developed.

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Conflicts of Interest

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