In the rapidly evolving landscape of cardiovascular disease research, the integration of next-generation technologies has led to a remarkable expansion in our understanding of disease pathogenesis. This theme issue of The American Journal of Pathology on “Advances in Understanding Cardiovascular Disease Pathogenesis” includes a series of review articles that highlight this dynamic landscape. These reviews expound on the transformative impact of omics technologies to uncover novel aspects of cardiovascular disease pathogenesis and facilitate the diagnosis and treatment of cardiovascular disease.

Unraveling Atherosclerotic Plaque Biology with Omics Profiling

Atherosclerotic cardiovascular disease, a chronic inflammatory disease that exhibits a complex, multifactorial etiology, drives both ischemic heart disease and ischemic stroke, globally the number 1 and number 5 leading causes of death, respectively.1 Atherosclerosis begins as a focal deposition of low-density lipoprotein particles into the arterial intima, resulting in a shift in endothelial phenotype to a permeable, proinflammatory state termed endothelial cell activation. Activated endothelial cells express cell adhesion molecules and secrete chemokines that drive monocyte recruitment into the intima to process the accumulated low-density lipoprotein. Modification of the low-density lipoprotein particles (eg, oxidation, glycation) causes dysregulated lipid accumulation in the macrophages, forming foam cells that can be visualized as fatty streaks in the artery. This lipid-driven chronic inflammatory response promotes vascular smooth muscle cells (vSMCs) to undergo a phenotypic transition and migrate from the media into the neointima.2 These smooth muscle cells (SMCs) can take on a fibroblast-like phenotype and deposit extracellular matrix proteins, resulting in the formation of a protective fibrous cap overlying the growing atheroma. As the plaque progresses, the microenvironment changes because of macrophage and SMC apoptosis or necroptosis, resulting in the formation of a necrotic core that exacerbates plaque inflammation and the potential for plaque rupture, a key driver of plaque-associated thrombosis.3 The fibrous cap limits the propensity for rupture and stabilizes the plaque. Plaque calcification, driven by SMCs that have taken on an osteochondrogenic phenotype, further reduces plaque stability, predisposing patients to a thrombotic event. Next-generation technologies have advanced our understanding of the phenotypic transitions that drive plaque formation and the underlying molecular mechanisms causing atherosclerotic cardiovascular disease pathogenesis.

The comprehensive review by Wu and Zhang4 peels back the layers of atherosclerotic plaques, spotlighting the instrumental role of omics technologies in exceeding the limitations of conventional histologic analysis. Beginning with genomics and epigenomics, the article sheds light on the underlying genetic predispositions and epigenetic modifications...
influencing atherosclerosis (Figure 1). Genetic studies have identified numerous loci associated with a heightened risk of developing atherosclerosis and related cardiovascular events. Variations in several genes contribute to the susceptibility and progression of atherosclerosis, impacting diverse pathways, such as lipid metabolism, inflammation, endothelial function, and vascular remodeling. Moreover, epigenetic modifications, such as DNA methylation and histone alterations, further regulate gene expression patterns linked to atherosclerosis, highlighting the dynamic interplay between genetics, environment, and epigenetics in shaping the disease process. Transitioning from these foundational aspects, the article intricately details transcriptomics, proteomics, metabolomics, and lipidomics analyses. These multi-omics approaches have provided a holistic view of the molecular landscape within atherosclerotic plaques, uncovering intricate gene expression patterns, protein signatures, and metabolic alterations.

Single-cell and spatial transcriptomics have collectively reshaped our comprehension of plaque inflammation by illuminating the intricate cellular heterogeneity and spatial organization within atherosclerotic lesions. These cutting-edge technologies, employed in tandem, unveil the diverse repertoire of immune cells, vascular constituents, and other cell types present within plaques, discerning unique transcriptional profiles of individual cell subsets involved in atherosclerosis.

Single-cell transcriptomics reveals distinct macrophage phenotypes, T-cell populations, endothelial cells, and more, each exhibiting specialized gene expression patterns linked to inflammation and plaque progression. Single-cell RNA sequencing has revealed diverse leukocyte populations within atherosclerotic plaques, including various macrophage clusters, T cells with diverse activation states, and specific subsets of dendritic cells and B cells. In parallel, spatial transcriptomics elucidates the spatial arrangement of these cell types and their associated gene expression within the plaque microenvironment, providing crucial context for understanding cellular interactions, localized inflammatory mediators, and the microanatomic distribution of key molecules within specific regions of the lesion.

Genomic Regulation of Endothelial Function in Atherosclerosis

Traditionally, the study of individual genetic variants associated with coronary artery disease risk has been painstaking and often insufficient in uncovering shared pathways...
regulated by multiple loci. The review by Pepin and Gupta highlights genetic variants with known roles in regulating endothelial function, as well as a novel approach, termed Perturb-seq, that uses a pooled clustered regularly interspaced short palindromic repeats (CRISPR)-based screen to perform an unbiased analysis of how the entire transcriptome affects specific cell phenotypes. Several genes associated with coronary artery disease risk regulate critical aspects of endothelial cell function, such as the endothelial response to shear stress, proinflammatory endothelial activation, and vasodilation. Flow patterns at atherosclerosis-prone regions critically regulate multiple aspects of endothelial phenotype, and several genes associated with processes important for endothelial shear sensing (eg, JCAD, KLF2/4) show genetic associations with coronary artery disease. Associations between FLT1 and MFGE8 and coronary artery disease highlight the important role of endothelial activation in orchestrating inflammatory gene expression, amplifying leukocyte recruitment, and advancing plaque development. Multiple regulators of vasodilation, including nitric oxide synthase, GUCY1A3, and PHACTR1, show genetic associations with coronary artery disease, although their relative role in plaque formation and tissue perfusion remains to be elucidated. Furthermore, studies using Perturb-seq allow for an unbiased analysis of genes regulating specific cell functions. For example, Perturb-seq studies looking for genes regulating low-density lipoprotein transcytosis identified an important role for activin receptor-like kinase 1 (Alk1). In addition, recent studies using Perturb-seq identified several genes associated with cerebral cavernous malformation (CCM2, KRIT1, and PDCD10) as critical regulators of endothelial phenotype in coronary artery disease, suggesting a potential link between common coronary artery disease variants and cerebral cavernous malformations (CCM)—related protective effects.

Unveiling Amino Acid Metabolism’s Link to Atherosclerotic Cardiovascular Disease

Metabolic dysregulation plays a pivotal role in driving atherosclerotic plaque formation, with aberrant lipid metabolism being a primary contributor. Although advancements in lipid-lowering therapies, such as statins and proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors, have significantly reduced plasma lipid levels and demonstrated efficacy in reducing cardiovascular death, coronary artery disease—associated morbidity and mortality has increased over the past decade. Beyond lipid-centric approaches, there is a growing recognition of the impact of amino acid metabolism in regulating atherosclerosis. The review by Anand et al has highlighted the role of amino acid metabolism in atherosclerosis pathogenesis, with a specific focus on arginine, branched-chain amino acids, glycine, aromatic amino acids, and selenocysteine. The review demonstrates the correlation between altered arginine metabolism, particularly the balance between its conversion to citrulline and ornithine, and the phenotypic shift in macrophages, suggesting their pivotal role in atherosclerosis progression and regression. Branched-chain amino acids, comprising leucine, isoleucine, and valine, have drawn attention because of their regulatory influence on metabolic health. Their intricate involvement in insulin signaling, mammalian target of rapamycin activation, and mitochondrial function presents a paradoxical relationship in atherosclerosis. Glycine, the simplest amino acid, emerges as a potential biomarker and intervention target in cardiovascular disease. Studies point toward its association with oxidative stress, inflammation, and metabolic syndrome, suggesting its role as an essential component in mitigating atherosclerosis.

Aromatic amino acids, such as tryptophan, tyrosine, and phenylalanine, offer a nuanced understanding of their metabolites’ influence on endothelial dysfunction and inflammation in atherosclerosis. The review delineates their roles in altering immune responses, increasing oxidative stress, and influencing plaque development, albeit acknowledging the complexity and sometimes contradictory nature of their effects. Selenocysteine, recognized for its potent antioxidant properties within selenoproteins, highlights the importance of oxidation-reduction homeostasis in atherosclerosis. The inclusion of selenocysteine’s role underlines the broader spectrum of amino acid metabolism impacting cardiovascular disease pathophysiology. Understanding the delicate balance of these amino acid pathways offers promising avenues for innovative therapeutic approaches in managing and preventing atherosclerosis and related cardiovascular diseases.

Deciphering the Many Faces of vSMCs

The diverse vasculopathies encompassed within the broader classification of cardiovascular disease present a highly intricate etiology, increasingly recognized to involve dysfunction across multiple organ systems. vSMCs have long been acknowledged for their significant role in various vasculopathies, including atherosclerotic cardiovascular disease, pulmonary hypertension, peripheral artery disease, and complications arising from surgical interventions, like vein graft stenosis, as well as restenosis in response to balloon angioplasty or stent placement. However, recent research underlines the inadequacy of our current understanding of vSMC function within various vasculopathies, emphasizing the need for deeper exploration and elucidation.

Brilliantly explained in the review by Ahmed et al, the advent and ingenious use of multiple -omics approaches have demonstrated that vSMC phenotypic plasticity is exceedingly complex, more so than previously thought. Using in vivo cell-fate tracking, coupled with genetic manipulation of specific target genes in a cell-specific manner, followed by single-cell transcriptomics, and
molecular imaging, researchers have greatly expanded our understanding of vSMC dynamics within various vasculopathies. These studies have shown that vSMCs can alter their phenotype in a far more wide-ranging manner than previously thought, with many of these phenotypes being identified in plaques in vivo. 27,28 Moreover, these studies demonstrate that the embryological origin of different vSMCs influences SMC phenotypic modulation and transcription factor expression in response to different extracellular stimuli. 29,30 Another discovery facilitated using advanced single-cell transcriptomics and epigenomics is that long-noncoding RNAs perform critical functions in the dynamic phenotypic plasticity of vSMCs, through facilitating myocardin binding to SMC-contractile gene promoters 31 or by forming a chromatin remodeling complex that perform repressive histone modifications at SMC-contractile gene promoters. 32 Taken together, the use of multiple -omics approaches has expanded our understanding of smooth muscle phenotypic modulation and identified multiple novel regulators of smooth muscle biology.

Chasing the Etiology of CAVD

Calcific aortic valve disease (CAVD) is increasingly becoming a significant risk factor for cardiovascular morbidity and mortality, with incidence for elderly patients expected to double by 2050. The disease is characterized by the calcification of the aortic valves within the heart and ranges in severity from aortic valve sclerosis to aortic valve stenosis, with the latter obstructing blood flow through the valve. As explained in the excellent review by Perez et al, 33 we have recently made great strides toward understanding the etiology of this disease and its progression from early to late stages. RNA-sequencing technologies also play a critical role in expanding our understanding of disease pathologies, including single-cell transcriptome analysis that can be leveraged to identify distinct subpopulations of cells within the diseased tissue. With respect to CAVD, this technology facilitated the identification of seven CAVD-enriched cell populations not observed in nondiseased samples; then, combined with molecular imaging techniques, researchers can visualize specific cell types within tissues and gain a greater understanding of the disease as a system. 34 Molecular imaging techniques have steadily and sometimes rapidly improved in recent years, and now allow for the observation of microcalcifications, which are presumed to precede the easily detected macrocalcifications, through pairing (18)F-sodium fluoride positron emission tomography imaging with a computed tomography scan. 35–37 This technology allows physicians to assess patients for microcalcifications before the disease progresses to valvular obstruction. A new alternative to the positron emission tomography/computed tomography scan combination is the use of near-infrared fluorescence intravital molecular imaging. This technology can be combined with probes for specific targets (e.g., hydroxyapatite for calcification) to allow for in vivo imaging of the probe target and allows researchers or physicians to visualize distinct cellular changes of early CAVD in vivo and validate targets identified through other methods. 38–40

Conclusions

As we navigate the many complexities encompassed within cardiovascular disease, it becomes increasingly evident that our ability to diagnose and intervene effectively hinges on cutting-edge technologies. This theme issue on “Advances in Understanding Cardiovascular Disease Pathogenesis” highlights how RNA-sequencing technologies, single-cell transcriptomics, chromatin mapping, proteomics, metabolomics, lipidomics, and molecular imaging have all converged to enable researchers to examine disease processes at an incredible level of detail and to facilitate the modeling of disease at a system, tissue, and cellular level. As our technology continues to advance and researchers implement ingenious methods to use that technology, our models of these diseases will grow in complexity to an extent that can only be rivaled by biology itself, thereby empowering researchers and physicians in their relentless pursuit to safeguard and enhance the lives of patients.

Author Contributions

C.B.D. and M.L.S. wrote the article; and A.W.O. revised the article. All authors contributed to the article and approved the submitted version.

Disclosure Statement

None declared.

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