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Epigenetic pathways in macrophages emerge as novel targets in atherosclerosis

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ABSTRACT

Atherosclerosis is a lipid-driven chronic inflammatory disorder. Monocytes and macrophages are key immune cells in the development of disease and clinical outcome. It is becoming increasingly clear that epigenetic pathways govern many aspects of monocyte and macrophage differentiation and activation. The dynamic regulation of epigenetic patterns provides opportunities to alter disease-associated epigenetic states. Therefore, pharmaceutical companies have embraced the targeting of epigenetic processes as new approaches for interventions. Particularly histone deacetylase (Hdac) inhibitors and DNA-methyltransferase inhibitors have long received attention and several of them have been approved for clinical use in relation to hematological malignancies. The key focus is still on oncology, but Alzheimer's disease, Huntington's disease and inflammatory disorders are coming in focus as well. These developments raise opportunities for the epigenetic targeting in cardiovascular disease (CVD). In this review we discuss the epigenetic regulation of the inflammatory pathways in relation to atherosclerosis with a specific attention to monocyte- and macrophage-related processes. What are the opportunities for future therapy of atherosclerosis by epigenetic interventions?

1. Introduction

1.1. Cardiovascular disease

Cardiovascular disease (CVD) is the most common cause of morbidity and mortality worldwide. 17.3 Million people are estimated to die of CVD annually, which is 30% of all deaths (WHO, 2015). Due to increased ageing and obesity prevalence, it is predicted that this number will further increase over time reaching 23.3 million in 2030 (Mathers and Loncar, 2006). The primary underlying cause of CVD is atherosclerosis, a slowly progressing chronic inflammatory disorder of the arteries. Atherosclerotic lesions develop in the intima of medium and large arteries and are characterized by the accumulation of lipids, the infiltration of monocytes, T cells and mast cells and the formation of a fibrous cap. This cap encloses the lesion and is made of collagen produced by smooth muscle cells (SMCs). Increased atherosclerotic plaque growth results in narrowing of the lumen, which may result in angina pectoris. Moreover, increased accumulation of lipids, macrophages and T cells may result in unstable lesions which ultimately can rupture and initiate thrombosis. Thrombosis can eventually result in acute myocardial infarction or stroke which are the main cause of CVD and overall death worldwide (Lozano et al., 2012). Risk factors for atherosclerosis include smoking, obesity, high blood pressure, diabetes and hyperlipidaemia. These factors induce vessel inflammation, thereby enhancing atherosclerosis.

1.2. Atherosclerosis Is an inflammatory disorder

Initiation of atherosclerosis develops as a result of a vascular injury, leading to a chronic inflammatory response over a long period of time (Ross, 1999). Vascular injury can result from shear stress, hyperlipidaemia and free radicals, which result in endothelial dysfunction. Together with high levels of circulating cholesterol and retention of oxidized low density lipoprotein (oxLDL) within the artery this triggers pro-inflammatory responses, which in turn are the first steps in the development of atherosclerotic plaques (Hansson and Libby, 2006; Hansson et al., 2002).

Inflammatory responses trigger local endothelial expression of adhesion molecules like vascular cell adhesion molecule 1 (VCAM-1). Circulating monocytes and T cells attach to activated endothelial cells and locally produced chemokines cause monocytes and T cells to migrate into the arterial intima (Boring et al., 1998; Gu et al., 1998). Once entered into the arterial tissue, monocytes will differentiate into macrophages in response to differentiation

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factors like macrophage colony-stimulating factor (M-CSF) (Johnson and Newby, 2009). When these macrophages subsequently engulf apoliporpotein B containing modified lipoproteins (e.g. oxLDL) they become foam cells (Glass and Witztum, 2001). Uptake of modified lipoproteins by macrophages is mediated by receptor-mediated endocytosis and pinocytosis, involving type A scavenger receptor and cluster of differentiation 36 (CD36) (Ashraf and Gupta, 2011; Kunjathoor et al., 2002). Stimulation of macrophages with those modified lipoproteins alters the inflammatory response. Yet, it still remains unclear whether these effects are pro- or anti-inflammatory and results appear to depend on experimental details. Indeed, Chavez-Sanchez et al. (2010) show that modified extracellular lipids can act via Toll-like receptor-2 and 4 (TLR-2 and TLR-4), thereby inducing inflammatory cytokine release from monocytes and macrophages. In contrast, Kannan et al. (2012) show that modified lipids rather block the cytokine response via TLR-2 and TLR-4 in human monocytes. In agreement with the latter observation, intracellular lipid accumulation dampens inflammation in peritoneal macrophages, an effect which is mediated by desmosterol (Spann et al., 2012). Intracellular accumulation of lipids does not result in down-regulation of scavenger receptors and thus leads to continued uptake and consequent foam cell formation (Rios et al., 2011). Accumulation of these lipid-laden macrophages in the vessel wall causes formation of so-called fatty streaks, the earliest signs of atherosclerotic disease. Not all fatty streaks develop into an atheroma, but they are precursors for plaques. Increase of macrophage intracellular lipids in combination with inflammatory signals will result in cytotoxicity and thus foam cell death. Release of their cellular content will further increase monocyte recruitment resulting in a vicious cycle. Therefore, foam cell formation is a crucial initiating step in the development of atherosclerotic lesions.

Small plaques increase in size by the continuous accumulation of inflammatory cells and extra-cellular lipids. Inflammatory cytokines like interleukin-1 (IL-1) and interferon-gamma (IFN-γ) together with growth factors (e.g. PDGF, thrombin) eventually cause SMCs to migrate from the media to the intima. Within the intima, SMCs are stimulated to produce collagen, elastin and proteoglycans resulting in fibrous cap formation. At this stage, foam cells are mostly located in the lipid core and T cells are found in clusters in the fibrous cap and shoulder regions of the lesion (Hansson et al., 2006). Expansion of the lipid core or increased SMC content results in narrowing of the lumen which can cause an occlusion. Furthermore, this can also cause thinning and eventually rupture of the fibrous cap resulting in thrombus formation (Fig. 1).

1.3. Macrophage function and polarization

As their name suggests (in Greek, *macros*=big and *phagein*=eat and thus 'macrophage=big eater'), the first function of macrophages to be identified by Metchnikoff was phagocytosis and microbial killing (Schmalstieg and Goldman, 2008). Whilst this is an important feature, macrophages are functionally much more complex and are involved in about every disease. In fact, they play a role in virtually all aspects of life; from development, homeostasis and tissue repair and to immunity (Jantsch et al., 2014).

Phenotypically, macrophages are phagocytic and express M-CSFR, CD11b, F4/80, CD64 and CD68. Yet the presence of these markers does not reveal their activation status. Indeed, macrophages are the most plastic cells of the hematopoietic system and in response to microenvironment stimuli they will adapt different polarization states. Whilst several efforts have been made to classify macrophages, the binary M1/M2 classification still remains the most used and offers a reductionist tool to describe extremes

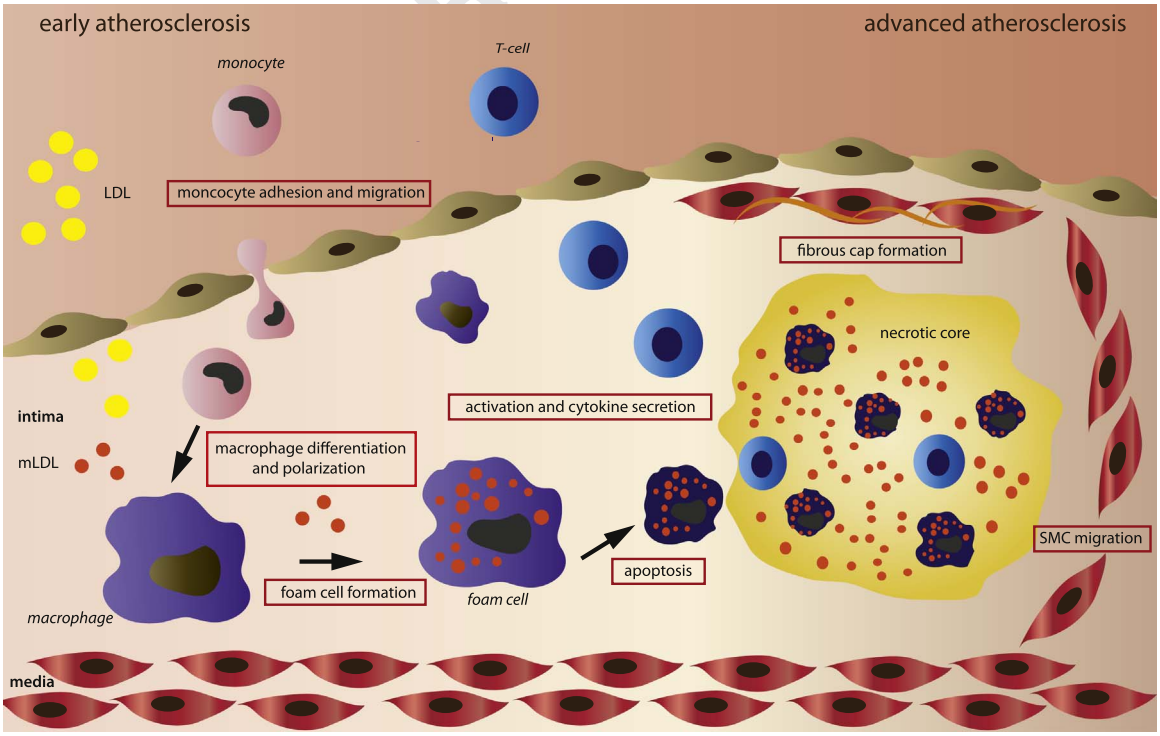

Fig. 1. Schematic representation of atherosclerotic plaque development. High levels of LDL get trapped in the intima of arteries leading to the formation of modified LDL (mLDL) and recruitment of monocytes. Monocytes adhere to the activated endothelium and migrate into the intima where they differentiate into macrophages. These macrophages scavenge modified lipid, causing foam cell formation. Increase uptake of lipids results in cytotoxicity, resulting in foam cell apoptosis and eventually necrotic core formation. Inflammatory cytokine secretion by macrophages and T cells promote further cellular activation and plaque growth and specific growth factors cause smooth muscle cell migration and fibrous cap formation. Indicated in red boxes are critical steps in atherogenesis. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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