Chapter 6
Smooth Muscle Cells in Diabetes Mellitus

Uma Nahar Saikia and Suvradeep Mitra

Abstract Diabetes mellitus is a multisystem systemic disease with significant morbidity and mortality. The morbidity is often contributed by the microvascular disease whereas the macrovascular disease is significantly associated with the mortality in the diabetic patients. The macrovascular disease, also known as diabetes-accelerated atherosclerosis is promoted by the interplay of multiple factors. These biochemical and molecular parameters predominantly affect the endothelial cells and the smooth muscle cells. Both these cells actively take part in the diabetes-accelerated atherosclerosis. The smooth muscle cells evidently proliferate, accumulate and show phenotype shift in diabetes-accelerated atherosclerotic lesions. These properties are studied mainly in the animal models and therapeutic drugs can be targeted to reduce these complications.

Keywords Diabetes • Atherosclerosis • Smooth muscle cells • Vascular disease • Hyperglycemia • Dyslipidemia

6.1 Introduction

Diabetes mellitus is a disease of quantitative or qualitative deficit of insulin resulting in a state of chronic hyperglycemia. Type 1 diabetes mellitus is characterized by the progressive destruction of the β cell population of the islets of Langerhans from an immunological process leading to a quantitative deficit of insulin whereas type 2 diabetes mellitus results from insulin resistance and subsequent loss of the islets. Different body systems are profoundly affected by diabetes, especially the vascular bed. Both the types of diabetes affect both the microvascular and the macrovascular compartments leading to significant morbidity and mortality. The microvascular compartment includes renal glomerulus, vasa nervorum of the peripheral nerves and retina leading to diabetic nephropathy, neuropathy and retinopathy or diabetic

U.N. Saikia, M.D. (✉) • S. Mitra
Department of Histopathology, Postgraduate Institute of Medical Education and Research,
Research Block A, 5th Floor, Chandigarh 160012, India
e-mail: umasaikia@gmail.com

triopathy if all the three compartments are involved. Macrovascular disease necessarily means diabetes-accelerated atherosclerosis involving the major vessels like aorta and its major branches. Diabetes leads to accelerated formation/progression of lesions of atherosclerosis affecting the vasculature. Myocardial ischemia and infarction, cardiovascular accident (stroke in common parlance) and limb ischemia or dry gangrene leading to limb amputation is the life-threatening or limb-threatening complications of the diabetic macrovascular disease. Needless to say that there has been a flurry of research around diabetes-accelerated atherosclerosis in recent times. The morphologic changes in the blood vessels followed by the pathobiology of the disease is discussed to understand the role of diabetes on the smooth muscle cells (SMCs).

6.2 Factors Affecting Smooth Muscle Cell Proliferation in Diabetes

Many biochemical factors are implicated in the SMC pathobiology. The main biochemical pathways involved are (1) polyol pathway, (2) hexosamine pathway, (3) advanced glycation/lipoxidation end-product (AGE/ALE) pathway and (4) protein kinase C (PKC) pathway [1]. These biochemical pathways portend in the generation of the reactive oxygen species (ROS) which forms the final common pathway [1]. Different studies have also shown that multiple parameters including chronic hyperglycemia, insulin resistance, hyperlipidemia etc. can affect the SMCs individually as well as concurrently. The basic pathogenetic scheme of the diabetes-accelerated atherosclerosis is depicted in Fig. 6.1.

6.3 Morphological Changes of Smooth Muscle Cells Secondary to Hyperglycemia and Dyslipidemia

The diabetic changes occur at a global scale. Variable research attempts have been made to pinpoint the site of the major brunt of the injury. Endothelial cell is undoubtedly one of the major sites to be inflicted with ROS. In addition to the endothelial cells, the micro and macrovasculature in the diabetic patients show smooth muscle cell (SMC) proliferation and morphological changes which help in the progression of atherosclerosis. The molecular biology of the SMCs in diabetes-accelerated atherosclerosis has been studied in the experimental models. In small animal models, infiltration of the monocytes followed by the activation and differentiation of these cells into lipid-loaded macrophages is seen in areas without pre-existing intimal thickening [2]. In culture, the most potent growth factors for SMCs are platelet derived growth factor B chain homodimer (PDGF-BB) and fibroblast growth factor-2 (FGF-2). SMC proliferation is also regulated by other factors i.e. components of
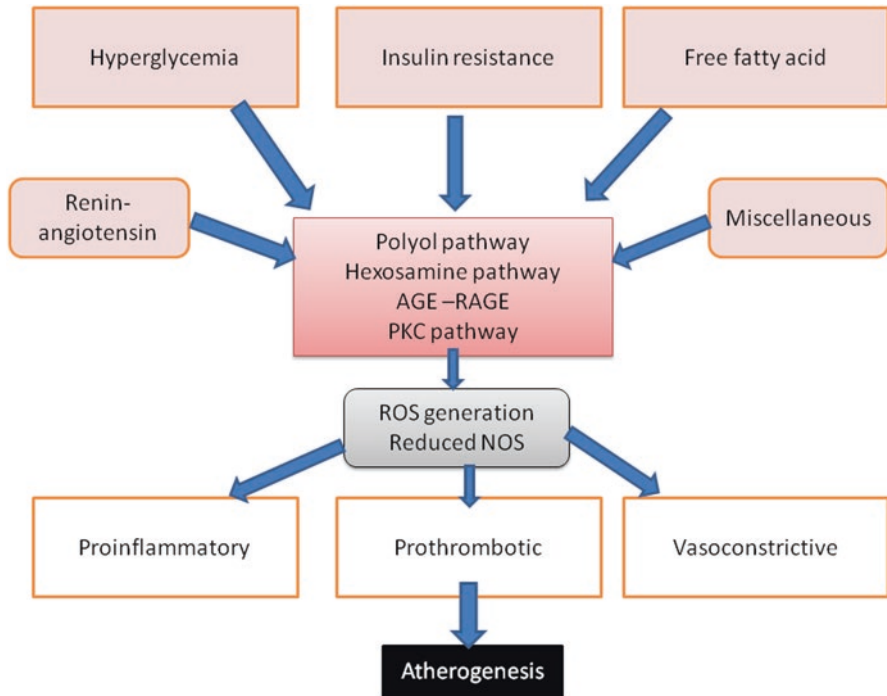
Fig. 6.1 Basic biochemical pathway of atherogenesis in diabetes

the extracellular matrix, and O2 tension [3]. Although these factors stimulate prolif-
eration of cultured SMCs, one should bear in mind that in vivo and ex vivo effects
may differ.

In humans, lipid-loaded macrophages are seen in areas with intimal thickening
which is smooth muscle cell mass followed by an increased accumulation of lipid-
loaded macrophages and extracellular lipid called as atheroma [4]. Formation of
"atheroma" (type IV lesion) means the accumulation of lipid laden macrophages in
the intima at a subendothelial location [4, 5]. The next step for progression of the
lesion is increased accumulation of smooth muscle cells (SMCs) in the intima and
formation of a fibroatheroma. "Fibroatheroma" (type V lesion) is the formation of a
fibrous cap over the atheroma with a central core containing the lipid laden macro-
phages [4, 5]. Many of these lipid laden macrophages die releasing their content
extracellularly forming the lipid rich core. The dead cells and their debris accumulate
in the core due to ineffective clearance (efferocytosis) [6] making the core material
more thrombogenic. The fibrous cap may rupture exposing the highly thrombogenic
core and causing "plaque rupture" or may remain stable with accumulation of more
material in the core thereby causing "progressive occlusion" of the vessel [7]. These
lesions can become destabilized, possibly by thinning of the SMC-rich fibrous cap
and/or increased macrophage death, leading to plaque rupture, thrombosis (Fig. 6.2a,
b), and the acute clinical manifestations of atherosclerosis [2, 8].
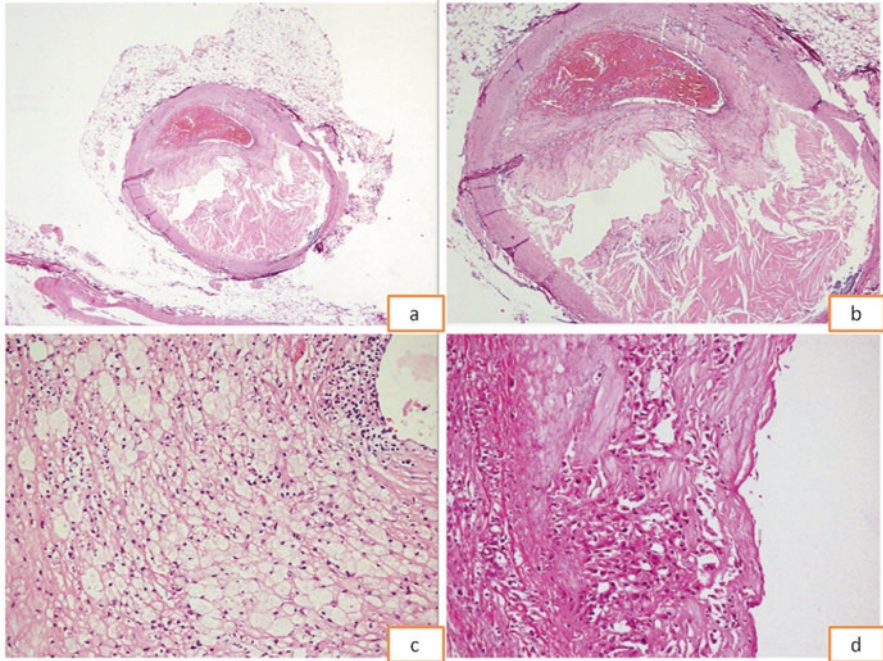
Fig. 6.2 (**a**) Scanner view of a medium sized artery (*Left* Anterior Descending Artery) in a patient with diabetes-accelerated atherosclerosis (Hematoxylin and Eosin, 20×); The artery is near-totally occluded. (**b**) The occlusion is because of the plaque rupture and thrombosis (Hematoxylin and Eosin, 40×); Note the fibrous cap is thinned out at one edge and numerous needle shaped cholesterol clefts in the atheroma core. (**c**) Numerous subintimal foam cell accumulation associated with sprinkling of the lymphocytes (Hematoxylin and Eosin, 200×). (**d**) Some of the foam cells show myoid type morphology with abundant deep eosinophilic cytoplasm (Hematoxylin and Eosin, 200×)

The atheroma formation necessarily begins with endothelial injury, a proinflammatory milieu and subsequent accumulation of subendothelial macrophages at the site of turbulence. The associated dyslipidemia promotes the accumulation of the lipid material within these macrophages. The circulating low density lipoprotein (LDL) particles in diabetes are small and dense. Diabetes associated hyper triglyceridemia often contributes to the generation of these small, dense LDLs. They offer higher penetration, increased susceptibility to oxidation and stronger avidity to the endothelium rendering them more atherogenic than the larger LDL particles [9]. The oxidation of the LDL particles is a crucial step as the oxidized LDL is antigenic and incites a chronic low grade inflammation at the site of atheroma formation with recruitment of the inflammatory cells which perpetuates the inflammation and subsequent endothelial injury by degranulation. Moreover, glycation of LDL increases the half life of LDL while the half life of the glycated high density lipoprotein (HDL) becomes shorter. In short, it means overproduction and perpetuation of LDL cholesterol and excess clearing of the protective HDL cholesterol. The atheroma formation occurs with the accumulation of lipid laden macrophages with subsequent intimal thickening. These macrophages recruit bone-marrow derived SMCs [10] as well as

promote the migration and proliferation of the intra-intimal and medial SMCs [11, 12]. Moreover, there is evidence that the macrophages themselves can differentiate and adopt myofibroblastic phenotype depending on the inflammatory milieu and the autocrine and paracrine factors released by the macrophages themselves and the endothelial cells. The increased accumulation of the SMCs in an atheroma portends the beginning of a fibroatheroma. The general belief of the migration of the medial SMCs, their proliferation and lack of apoptosis in the formation of fibroatheroma has been challenged by some authors in animal studies. Imperative to say, that different growth factors play roles in the formation of the fibrous cap.

The SMCs secrete collagen required for the formation of the fibrous cap strengthening the cap architecture. The increased apoptosis of the SMCs promote plaque rupture in two important ways. The first one is due to the relative lack of the collagen which is produced and secreted by the SMCs. The other cause is due to the release of pro-inflammatory cytokines related to the myocyte death which potentiates the instability of the plaque. The monocyte-macrophage system plays a crucial role in the plaque stabilization by influencing cell death of the SMCs. The advanced atherosclerotic lesions can undergo remodeling by an increase in the SMC content and reduction of the macrophage content as shown in the animal models. Also HDL can bring about similar changes and stabilize an atherosclerotic plaque [13]. The SMCs are recruited from the media and they migrate and accumulate in the vicinity of the plaque. The SMCs, native to the intima also undergo proliferation and accumulate in the atheromatous plaque. Some of these SMCs take up and accumulate the lipid within them similar to the foamy macrophages [14]. In fact, Katsuda et al. showed that the majority of the cellularity in the early atherosclerotic plaque is contributed by the SMCs. The foam cells also are mostly derived from the SMCs rather than from the macrophages (Fig. 6.2c, d), as was demonstrated by the immunohistochemistry [14]. These SMCs behave like the foamy macrophages and also undergo apoptotic death releasing the lipid material extracellularly. The extracellular lipid alongside the cellular debris forms the necrotic core. In addition, the SMCs also tend to contain this necrotic core within the subintima by forming a fibrous cap over it. This fibrous core is formed by the direct presence of the SMCs as well as by the secretion of collagen and elastin by the SMCs [7, 15].

Functionally, the SMCs can be divided into resident SMCs and migrating SMCs. The resident SMCs are native to the intima and is found normally within the intima, whereas the migrating SMCs are derived from the media and can only be seen in progressive atherosclerosis. The migration of the SMCs is stimulated by different mitogenic factors. The migrating and resident SMCs also receive proliferating signal to cause progression of the atherosclerotic process. Morphologically or ultrastructurally, these SMCs can be myofilament-rich or rough endoplasmic reticulum (RER)-rich. The myofilament-rich SMC is RER-poor and vice versa. The former serves a more contractile function and the later has synthetic functions. The contractile phenotype is also known as differentiated phenotype and is commonly found in the healthy blood vessels whereas the non-contractile/synthetic phenotype predominates in the diseased blood vessels [16]. It has been shown in animal models that the SMCs in the diabetes-accelerated atherosclerosis show a synthetic phenotype rather than a contractile phenotype along with a switch in the expression of the actin

isoform [16, 17]. The contractile alpha smooth muscle isoform converts to a non-muscle beta isoform in diabetes-accelerated atherosclerosis [17]. The advanced lesions of atherosclerosis (type III onwards) show visible ultrastructural difference [4, 5]. The basement-membrane around these SMCs is very thick giving it the name of basement-membrane-rich or pancake-like cell [18]. The diabetic SMCs show an abundance of cytoplasm and RERs on ultrastructure alongside an increased amount of extracellular material [17]. These phenotypic switch in diabetes-accelerated atherosclerosis is also termed as "phenotypic modulation" [17]. Moreover, studies have also pointed out a vascular bed specific remodeling in diabetes and differential phenotype of SMCs in different vascular beds in animal studies. As for example, the coronary SMCs are found to down regulate the expression of the contractile proteins with altered interaction with the extracellular matrix (ECM) as compared to the aortic SMCs. The phenotypic variation of the diabetic SMCs is also described in other studies. One such study concluded that the human SMCs isolated from the diabetic patients show a significantly higher rate of proliferation, adhesion and migration in addition to abnormal morphology in the culture medium [19]. This property of the SMCs is termed as "vascular hyperreactivity" by some authors and a change in the subcellular calcium ion distribution in activated SMCs has been postulated as one of the causes to bring about these changes [20]. The enzyme content (endothelial Nitric oxide synthetase), intracellular guanosine monophosphate (cGMP) levels also change in the diabetic SMCs leading to the hyperreactivity of the SMCs in the diabetic patients [16]. Indeed, calmodulin-stimulated cyclic nucleotide phosphodiesterase gets accumulated in the SMCs of both the phenotypes and takes part in the SMC proliferation and recruitment [21, 22].

It is also important to note that the SMCs in diabetic vessels do not show uniform phenotypic and/or functional change. In a seminal study by Boor et al., the enzymatic activity of the SMCs in the plaque region or underlying the plaque had been found to be different than the enzymatic activity in the vicinity. An isoenzyme of glutathione-S-transferase (GST), known as hGSTA4-4 is known to be associated with detoxification of the generated intracellular ROS [23]. Preferential expression of this enzyme in the SMCs underneath the plaque substantiates the involvement of SMCs in the pathogenesis of the diabetes-accelerated atherosclerosis. In addition, the role of ROS in the pathogenesis is also highlighted.

6.4   Biochemical and Pathobiologic Basis of Smooth Muscle Cell Proliferation in Diabetes

Despite the general and uniform agreement that SMCs proliferate and accumulate in diabetes-accelerated atherosclerosis, the true pathobiology of SMC induction is yet not elucidated. Many factors namely hyperglycemia, insulin, AGE, triglycerides and non-esterified fatty acids, hypertension and renin-angiotensin system and different paracrine molecules are all implicated with contradictory results in different studies. Similarly, the studies highlighting the factors affecting the stability of the

plaque are also not met with consensus agreement. Probably the SMC induction and plaque stability are the functions of multiple inter-related factors.

Different studies have concluded the effects of hyperglycemia on SMC proliferation differently. This ranges from stimulatory effect to no effect to inhibitory effect. However, few studies have shown that the glucose consumption by the SMCs in diabetes remains very high, so much so that they can be almost compared to the tumour cells in terms of glucose hunger. However, unlike the endothelial cells, the SMCs use glycolytic pathway for ATP generation even under aerobic condition, a paradoxical condition known as "aerobic glycolysis". Energetically infidel, aerobic glycolysis protects the SMCs from the oxidative stress [24]. There are some studies proposing the theory of induction of proliferation and accumulation of the SMCs by chronic hyperglycemia [16, 25–27]. Application of different inhibitor drugs on animal subjects have shown consequent blockade of the SMC proliferation and accumulation. For example, epalrestat, the inhibitor of aldose reductase enzyme (key enzyme of polyol pathway) has abolished the proliferative and migratory phenotypic and functional switch in diabetic animals proving the role of polyol pathway in the pathogenesis [27]. Similarly, the anti-insulin drugs are also found to nullify the SMC chemokinesis substantiating the role of free radical pathway in SMC pathobiology [26]. Advanced glycation end products (AGE)s are found to cause oxidative stress in the SMCs by the AGE-RAGE (receptor of AGE) interaction and the growth stimulatory effect of this ligand-receptor interaction. Also, the altered cell-cell and cell-matrix interaction promoted by the AGEs or ALEs (advanced lipoxidation end products) cause an aberrancy of cellular function. On the contrary, the stimulatory effect of chronic hyperglycemia on SMC has been challenged by a few authors [12, 28]. In this context, the study by Peiro et al. is noteworthy. These authors had shown a death promoting effect of chronic hyperglycemia on the SMCs by the activation of necrotic pathway. Moreover, hydrogen peroxide has been found to play a pivotal role in this necrotic cell death, as catalase enzyme had been found to abolish these effects. This necrotic cell death promotes the changes of diabetic vasculopathy [28]. Morphologically, the changes of both diabetic vasculopathy and accelerated atherosclerosis are well documented in the same organ. Hence, probably, chronic hyperglycemia has a complex interaction with the SMCs and the smooth muscle changes are function of multiparametric interaction.

There is evidence of lesional triglyceride (TG) and non-esterified fatty acids (NEFA) promoting SMC migration and proliferation in the recent literature. The lipoprotein lipase, an essential enzyme in the degradation of the TG is increased in atheromatous lesions and is released by the SMCs and the macrophages in an atheromatous plaque. Recent evidence suggests that the insulin resistance has adverse effects on both the endothelium and the platelets via the downregulation of the PI3K/AKT and IRS-1/AKT pathways ultimately promoting an imbalance of nitric oxide (NO) and reactive oxygen species (ROS). However, the role of insulin or insulin-resistance on SMCs is not well established [11].

The nitrergic pathway, intracellular guanosine monophosphate (cGMP) and ROS are considered to be the final common pathway linking all the other metabolic pathways in diabetes mellitus, namely the polyol, hexosamine, AGE and protein kinase

C (PKC) pathways [1, 29]. Many studies including the study by Pandolfi et al. had shown the upregulation of the intra-endothelial and intra-SMC nitric oxide synthetase (NOS) activity along with a concurrent fall in cGMP and rise in superoxide production in diabetic animals [16].

The renin-angiotensin pathway, hypertension and the paracrine factors may also affect the SMCs, though convincing evidences are still lacking. Similarly, no such factors can be implicated for the plaque instability [11].

From this discussion, it is evident that diabetes causes SMC dysfunction or diabetes is a state of altered function for SMCs. This SMC dysfunction is attributed to variable factors but hyperglycemia, insulin resistance and dyslipidemia all play roles in this. These factors are also keys to the vascular damage and subsequent atherogenesis. All these three factors promote oxidative damage to the endothelium bringing about a vasoconstrictive, pro-inflammatory and prothrombotic state all of which stimulate atherogenesis independently or in a combined manner. So, in brief, diabetes is a state of phenotypic modulation and dysfunction of the SMCs [30].

6.5 Significance of the Smooth Muscle Cell Changes in Diabetes

Once the morphological and functional changes in SMCs occur, consequently the imminent question that looms large is "Do these changes reflect mere research-related jargon or they have any diagnostic, prognostic or therapeutic value"? In simple words, what is the clinical relevance of these SMC changes in relation with diabetes which is necessarily twofold in clinical practice. The first one is prognostic and the second one is therapeutic. Currently, the diagnosis of diabetes-accelerated atherosclerosis is more clinical and comes from the end-organ-damage related signs and symptoms. The above-mentioned pathways are experimental studies either in cell culture or in animal models.

The involvement of the SMCs dictate the development, progression and the complications of the diabetes associated macrovascular disease. It is evident from the above-mentioned discussion that the SMCs undergo a series of phenotypic and functional changes in the diabetic setting. These changes may involve preferential vascular beds and can have regional preferences as well. The basic underlying change of the SMCs in diabetes is the proliferation and accumulation leading to the formation of the atheroma. Moreover, the SMCs undergo foam cell transformation in the early stage. The next step is progression of the atheroma into fibroatheroma which is brought about by the secretion of collagen by the SMCs. The complications can occur either in the form of plaque rupture or plaque vulnerability or stability of the plaque leading to luminal occlusion. Dissecting aneurysm and vasculopathy are also known complications of diabetic macrovascular disease. Although the exact mechanism of the plaque rupture is not well elucidated, it is probably caused by a thinner fibrous cap. The thinner fibrous cap can be formed by a lesser collagen

secretion by SMCs and this diminution of the collagen secretion could be because of less proliferation and/or accumulation of the SMCs or their death by apoptosis or a functional defect of the accumulated SMCs. The SMC apoptosis also promotes dissecting aneurysm and diabetic vasculopathy.

The therapeutic significance of the SMC involvement is again twofold. According to the previous concept, the endothelial cells are believed to be the key mediators in the diabetic vascular diseases. However, the recent concept proves the fact that SMCs play a major role in diabetic diseases independent of or dependent on the endothelial injury. This necessitates targeting the SMCs with or without the endothelial cell activation. As for example, the foam cells in early atherosclerosis are experimentally targeted as their presence and trafficking promotes the progression. As the foam cell population consists of both the macrophages and SMCs, they become the natural therapeutic targets [31]. SMC proliferation and migration along with the biosynthetic activity is also targeted for causing plaque regression [32]. Endoglin receptor modulator modulates the mural cell adhesion and their proliferation and is found to be beneficial in atherosclerosis [33, 34]. Secondly, the underlying mechanisms of the endothelial dysfunction have been postulated. The individual modulators of these postulated pathways are found to have beneficial effects (anti-proliferative and anti-migratory) on the SMCs in the animal models. Targeting these pathways in the human beings may prove to have therapeutic effects. Naturally, the antioxidants and free radical scavengers, anti-PKC agents, aldose reductase inhibitors are all effective [32]. The role of epigenetic modifiers and micro RNAs in this aspect are also being evaluated [35, 36].

6.6   Conclusion

In short, diabetes promotes a state of smooth muscle cell dysfunction and proliferation coexisting with its phenotypic changes. These changes probably occur as a result of the complex interaction of multiple biochemical parameters. This constitutes the effector mechanism of the formation, progression and the complications of the atherosclerotic plaque. A detailed knowledge of such underlying pathogenesis may help in the development of the newer targeted therapies in diabetes associated accelerated atherosclerosis.

References

 1. Brownlee M (2001) Biochemistry and molecular cell biology of diabetic complications. Nature 414(6865):813–820. doi:10.1038/414813a
 2. Ross R (1993) The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature 362(6423):801–809. doi:10.1038/362801a0
 3. Berk BC, Abe JI, Min W, Surapisitchat J, Yan C (2001) Endothelial atheroprotective and anti-inflammatory mechanisms. Ann N Y Acad Sci 947:93–109. discussion 109-111

4. Stary HC, Chandler AB, Dinsmore RE, Fuster V, Glagov S, Insull W Jr, Rosenfeld ME, Schwartz CJ, Wagner WD, Wissler RW (1995) A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis. Am Heart Assoc Circ 92(5):1355–1374

5. Stary HC, Chandler AB, Dinsmore RE, Fuster V, Glagov S, Insull W Jr, Rosenfeld ME, Schwartz CJ, Wagner WD, Wissler RW (1995) A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. Arterioscler Thromb Vasc Biol 15(9):1512–1531

6. Libby P, Ridker PM, Hansson GK (2011) Progress and challenges in translating the biology of atherosclerosis. Nature 473(7347):317–325. doi:10.1038/nature10146

7. Bentzon JF, Otsuka F, Virmani R, Falk E (2014) Mechanisms of plaque formation and rupture. Circ Res 114(12):1852–1866. doi:10.1161/CIRCRESAHA.114.302721

8. Glass CK, Witztum JL (2001) Atherosclerosis. The road ahead. Cell 104(4):503–516

9. Hodis HN, Mack WJ, Dunn M, Liu C, Selzer RH, Krauss RM (1997) Intermediate-density lipoproteins and progression of carotid arterial wall intima-media thickness. Circulation 95(8):2022–2026

10. Fledderus JO, van Oostrom O, de Kleijn DP, den Ouden K, Penders AF, Gremmels H, de Bree P, Verhaar MC (2013) Increased amount of bone marrow-derived smooth muscle-like cells and accelerated atherosclerosis in diabetic apoE-deficient mice. Atherosclerosis 226(2):341–347. doi:10.1016/j.atherosclerosis.2012.11.017

11. Askari B, Renard CB, Bornfeldt KE (2002) Regulation of smooth muscle cell accumulation in diabetes-accelerated atherosclerosis. Histol Histopathol 17(4):1317–1328

12. Suzuki LA, Poot M, Gerrity RG, Bornfeldt KE (2001) Diabetes accelerates smooth muscle accumulation in lesions of atherosclerosis: lack of direct growth-promoting effects of high glucose levels. Diabetes 50(4):851–860

13. Rong JX, Li J, Reis ED, Choudhury RP, Dansky HM, Elmalem VI, Fallon JT, Breslow JL, Fisher EA (2001) Elevating high-density lipoprotein cholesterol in apolipoprotein E-deficient mice remodels advanced atherosclerotic lesions by decreasing macrophage and increasing smooth muscle cell content. Circulation 104(20):2447–2452

14. Katsuda S, Boyd HC, Fligner C, Ross R, Gown AM (1992) Human atherosclerosis. III Immunocytochemical analysis of the cell composition of lesions of young adults. Am J Pathol 140(4):907–914

15. Gautier EL, Huby T, Witztum JL, Ouzilleau B, Miller ER, Saint-Charles F, Aucouturier P, Chapman MJ, Lesnik P (2009) Macrophage apoptosis exerts divergent effects on atherogenesis as a function of lesion stage. Circulation 119(13):1795–1804. doi:10.1161/CIRCULATIONAHA.108.806158

16. Pandolfi A, Grilli A, Cilli C, Patruno A, Giaccari A, Di Silvestre S, De Lutiis MA, Pellegrini G, Capani F, Consoli A, Felaco M (2003) Phenotype modulation in cultures of vascular smooth muscle cells from diabetic rats: association with increased nitric oxide synthase expression and superoxide anion generation. J Cell Physiol 196(2):378–385. doi:10.1002/jcp.10325

17. Etienne P, Pares-Herbute N, Mani-Ponset L, Gabrion J, Rabesandratana H, Herbute S, Monnier L (1998) Phenotype modulation in primary cultures of aortic smooth muscle cells from streptozotocin-diabetic rats. Differ Res Biol Divers 63(4):225–236. doi:10.1111/j.1432-0436.1998.00225.x

18. Stary HC (1990) The sequence of cell and matrix changes in atherosclerotic lesions of coronary arteries in the first forty years of life. Eur Heart J 11(Suppl E):3–19

19. Faries PL, Rohan DI, Takahara H, Wyers MC, Contreras MA, Quist WC, King GL, Logerfo FW (2001) Human vascular smooth muscle cells of diabetic origin exhibit increased proliferation, adhesion, and migration. J Vasc Surg 33(3):601–607. doi:10.1067/mva.2001.111806

20. Fleischhacker E, Esenabhalu VE, Spitaler M, Holzmann S, Skrabal F, Koidl B, Kostner GM, Graier WF (1999) Human diabetes is associated with hyperreactivity of vascular smooth muscle cells due to altered subcellular Ca2+ distribution. Diabetes 48(6):1323–1330

21. Rybalkin SD, Bornfeldt KE, Sonnenburg WK, Rybalkina IG, Kwak KS, Hanson K, Krebs EG, Beavo JA (1997) Calmodulin-stimulated cyclic nucleotide phosphodiesterase (PDE1C) is induced in human arterial smooth muscle cells of the synthetic, proliferative phenotype. J Clin Invest 100(10):2611–2621. doi:10.1172/JCI119805

22. Rybalkin SD, Rybalkina I, Beavo JA, Bornfeldt KE (2002) Cyclic nucleotide phosphodiesterase 1C promotes human arterial smooth muscle cell proliferation. Circ Res 90(2):151–157

23. Boor PJ, Yang Y, Gong B (2006) Role of the media in vascular injury: atherosclerosis and dissection. Toxicol Pathol 34(1):33–38. doi:10.1080/01926230500369907

24. Morrison AD, Berwick L, Orci L, Winegrad AI (1976) Morphology and metabolism of an aortic intima-media preparation in which an intact endothelium is preserved. J Clin Invest 57(3):650–660. doi:10.1172/JCI108321

25. Hall JL, Matter CM, Wang X, Gibbons GH (2000) Hyperglycemia inhibits vascular smooth muscle cell apoptosis through a protein kinase C-dependent pathway. Circ Res 87(7):574–580

26. Watson PA, Nesterova A, Burant CF, Klemm DJ, Reusch JE (2001) Diabetes-related changes in cAMP response element-binding protein content enhance smooth muscle cell proliferation and migration. J Biol Chem 276(49):46142–46150. doi:10.1074/jbc.M104770200

27. Yasunari K, Kohno M, Kano H, Yokokawa K, Horio T, Yoshikawa J (1995) Aldose reductase inhibitor prevents hyperproliferation and hypertrophy of cultured rat vascular smooth muscle cells induced by high glucose. Arterioscler Thromb Vasc Biol 15(12):2207–2212

28. Peiro C, Lafuente N, Matesanz N, Cercas E, Llergo JL, Vallejo S, Rodriguez-Manas L, Sanchez-Ferrer CF (2001) High glucose induces cell death of cultured human aortic smooth muscle cells through the formation of hydrogen peroxide. Br J Pharmacol 133(7):967–974. doi:10.1038/sj.bjp.0704184

29. Aronson D, Rayfield EJ (2002) How hyperglycemia promotes atherosclerosis: molecular mechanisms. Cardiovasc Diabetol 1:1

30. Paneni F, Beckman JA, Creager MA, Cosentino F (2013) Diabetes and vascular disease: pathophysiology, clinical consequences, and medical therapy: Part I. Eur Heart J 34(31):2436–2443. doi:10.1093/eurheartj/eht149

31. Moore KJ, Sheedy FJ, Fisher EA (2013) Macrophages in atherosclerosis: a dynamic balance. Nat Rev Immunol 13(10):709–721. doi:10.1038/nri3520

32. Schwartz CJ, Valente AJ, Sprague EA, Kelley JL, Cayatte AJ, Mowery J (1992) Atherosclerosis. Potential targets for stabilization and regression. Circulation 86(6 Suppl):III117–III123

33. Jamkhande PG, Chandak PG, Dhawale SC, Barde SR, Tidke PS, Sakhare RS (2014) Therapeutic approaches to drug targets in atherosclerosis. Saudi Pharm J: SPJ: Off Publ Saudi Pharm Soc 22(3):179–190. doi:10.1016/j.jsps.2013.04.005

34. Rossi E, Smadja DM, Boscolo E, Langa C, Arevalo MA, Pericacho M, Gamella-Pozuelo L, Kauskot A, Botella LM, Gaussem P, Bischoff J, Lopez-Novoa JM, Bernabeu C (2016) Endoglin regulates mural cell adhesion in the circulatory system. Cell Mol Life Sci: CMLS 73(8):1715–1739. doi:10.1007/s00018-015-2099-4

35. Loyer X, Mallat Z, Boulanger CM, Tedgui A (2015) MicroRNAs as therapeutic targets in atherosclerosis. Expert Opin Ther Targets 19(4):489–496. doi:10.1517/14728222.2014.989835

36. Natarajan R (2011) Drugs targeting epigenetic histone acetylation in vascular smooth muscle cells for restenosis and atherosclerosis. Arterioscler Thromb Vasc Biol 31(4):725–727. doi:10.1161/ATVBAHA.111.222976