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Controlling the angiogenic switch in developing atherosclerotic plaques: Possible targets for therapeutic intervention

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Abstract

Plaque angiogenesis may have an important role in the development of atherosclerosis. Vasa vasorum angiogenesis and medial infiltration provides nutrients to the developing and expanding intima and therefore, may prevent cellular death and contribute to plaque growth and stabilization in early lesions. However in more advanced plaques, inflammatory cell infiltration, and concomitant production of numerous pro-angiogenic cytokines may be responsible for induction of uncontrolled neointimal microvessel proliferation resulting in production of immature and fragile neovessels similar to that seen in tumour development. These could contribute to development of an unstable haemorrhagic rupture-prone environment. Increasing evidence has suggested that the expression of intimal neovessels is directly related to the stage of plaque development, the risk of plaque rupture, and subsequently, the presence of symptomatic disease, the timing of ischemic neurological events and myocardial/cerebral infarction. Despite this, there is conflicting evidence regarding the causal relationship between neovessel expression and plaque thrombosis with some in vivo experimental models suggesting the contrary and as yet, few direct mediators of angiogenesis have been identified and associated with plaque instability in vivo.

In recent years, an increasing number of angiogenic therapeutic targets have been proposed in order to facilitate modulation of neovascularization and its consequences in diseases such as cancer and macular degeneration. A complete knowledge of the mechanisms responsible for initiation of adventitial vessel proliferation, their extension into the intimal regions and possible de-novo synthesis of neovessels following differentiation of bone-marrow-derived stem cells is required in order to contemplate potential single or combinational anti-angiogenic therapies. In this review, we will examine the importance of angiogenesis in complicated plaque development, describe the current knowledge of molecular mechanisms of its initiation and maintenance, and discuss possible future anti-angiogenic therapies to control plaque stability.

Introduction

According to a World Health Organization Fact Sheet (EURO/03/06) cardiovascular disease (CVD) is the number one killer in Europe, with heart disease and stroke being the major cause of death in all 53 Member States. Figures show that 34,421 (23% of all non-communicable diseases) of Europeans died from CVD in 2005. The report also highlighted the fact that there is approximately a 10-fold difference in premature CVD mortality between Western Europe and countries in Central and Eastern Europe with a higher occurrence of CVD amongst the poor and vulnerable. Although improvements in understanding have helped to reduce the number of Western European dying from CVD and related diseases further advances will require a clearer understanding of the pathobiological mechanisms responsible for the development of stroke,

atherosclerosis and myocardial infarction. Approximately 75% of acute coronary events and 60% of symptomatic carotid artery disease are associated with disruption of atherosclerotic plaques [1]. *In 1971, Folkman* [2]*introduced the concept of angiogenesis as a necessity for tumour growth. Its importance in other pathological conditions, including, atherosclerosis, myocardial infarction and stroke was later realized* [3, 4]. Angiogenesis is the formation of new blood vessels from a pre-existing vascular network, and is an important phenomenon in physiological situations such as embryonic development and wound healing as well as in pathological conditions like diabetic retinopathy, rheumatoid arthritis, tumor progression and atherosclerosis. Endothelial cells (EC) which line the inside of blood vessels are the target cells of angiogenic regulators. Stimulated EC undergo metabolic modifications associated with the main steps of angiogenesis i.e. production of matrix metalloproteinases that degrade the basement membrane and extracellular matrix (ECM); stimulation of EC migration and proliferation; secretion of collagen and differentiation resulting in sprout formation and ultimately, formation of new blood vessels [3, 5].

Angiogenesis in cardio- and cerebrovascular disease: Evidence for it as a major determinant of plaque growth, instability and rupture

Relationship between inflammation and angiogenesis in promotion of plaque development?

The atherosclerotic process is often initiated before adulthood, when cholesterol-containing low-density lipoproteins accumulate in the intima and activate the endothelium. Leukocyte adhesion molecules and chemokines promote recruitment of monocytes and T cells. Monocytes can differentiate into macrophages and up-regulate pattern recognition receptors, including scavenger receptors and toll-like receptors. Scavenger receptors mediate lipoprotein internalization, which leads to foam-cell formation. Toll-like receptors transmit activating signals that lead to the release of cytokines, proteases, and vasoactive molecules, and are considered to be an important link between inflammation and cardiovascular disease [25]. Deficiency of toll-like receptor 4 (TLR-4) protein leads to a reduction in macrophage recruitment in association with reduced cytokine and chemokine levels [26]. T cells in lesions recognize local antigens and mount T helper-1 responses with secretion of pro-inflammatory cytokines that contribute to local inflammation and growth of the plaque [27].

VV density, their proliferation and medial-intimal infiltration, and concurrent adventitial inflammation are strongly associated with advanced lesions [28]. Plaque neovascularization correlated with the extent of inflammation in hypercholesterolemic apoE mice and inhibition of vessel formation reduced macrophage accumulation and plaque progression [22]. Similarly, transfection with murine soluble VEGF-R1 inhibited early inflammation and late neointimal formation, again suggesting that as neovessels are generated, so the inflammatory response is perpetuated in a continuous cycle [29]. Inflammatory infiltrates enhance recruitment of monocytes, secrete matrix metalloproteinases and increase the expression of γ-interferon (from t-lymphocytes) which may weaken the fibrous cap; similarly, they can induce synthesis of angiogenic tissue angiotensin-converting enzyme, growth factors, interleukin-8 and tissue factor [15]. The importance of the inflammatory response was demonstrated in vivo where oral treatment of apoE-/LDL-double knockout mice with the anti-inflammatory compound 3-deazaadenosine prevented lesion formation [30]. A strong correlation has been shown between macrophage infiltration, intraplaque haemorrhage and rupture-prone thin-cap lesions with high microvessel density, whilst these features are not common in calcified or hyalinized human arterial plaques, suggesting a strong link between neovascularization, inflammation and thrombosis [15, 31]. The phenotype of plaque neovessels could also be important in determining plaque stability, for example, new vascular networks, and immature vessels with poor integrity and no smooth muscle cell/pericyte coverage would likely act as sites for inflammatory cell infiltration, inflammatory cell leakage and intraplaque haemorrhage respectively [22]. The correlation of focal collections of inflammatory cells with areas of intraplaque neovascularization and haemorrhage, suggests that release of growth factors and cytokines by macrophages and leukocytes may have a key role in modulating the vascularization process [8]. Evidence for the existence of hotspots or "neovascular milieu" was found in lesions from ApoE-/- mice where the density of VV was highly correlated with the presence of inflammatory cells rather than plaque size, whilst deposition of RBC membranes within the necrotic core of plaques also leads to an increase in macrophage infiltration and therefore may further potentiate the inflammatory response [22, 23].

Hypercholesterolaemia may be associated with proliferation of VV in coronary and carotid vessels at early stages of plaque development. Williams JK [32] first demonstrated that the presence of atherosclerosis in hypercholesterolemic monkeys induced an increase in blood flow through the VV and plaque regression caused by removal of the high lipid diet reduced the VV concentration and blood flow to the coronary media and intima. High cholesterol levels are associated with increased serum VEGF expression and may cause up-regulation of growth factor receptors on both endothelial and smooth muscle cells [33]. Furthermore, oxidized LDL (ox-LDL) generated in response to pro-oxidative cellular changes can exacerbate the inflammatory response, since engulfment of intact apoptotic cells was reduced in

the presence of ox-LDL and in its absence, rapid phagocytosis suppressed macrophage inflammatory cytokine release, suggesting a link between high lipid levels, inflammation and possibly angiogenesis [33]. In vitro studies have demonstrated that stimulation of HUVEC with ox-LDL up-regulates adhesion molecules (including ICAM-1, E-selectin and P-selectin), inflammatory proteins including Il-6, thrombotic factors including tissue factor and remodeling proteins such as MMP-2 and MMP-9, many of which are also stimulators of angiogenesis [34].

Medial and intimal thickening induced by hypercholesterolemia may result in a limited supply of oxygen and nutrients reaching these areas from either the lumen and/or VV, resulting in a hypoxic environment. Since the major outcome of hypoxia is increased vascularization, intraplaque vessels may proliferate in association with this potent stimulus. Hypoxia-inducible factor (HIF-1) is expressed in hypoxic regions of expanding and developing plaques, and directs migration of EC towards the hypoxic environment via direct HIF-1 binding the regulatory gene of VEGF and subsequent induction of VEGF transcription [35]. VEGF is a potent angiogenic growth factor, stimulating EC mitogenesis and blood vessel formation via activation of intracellular signalling intermediates including mitogen-activated protein kinase 1/2 (MAPK1/2) and src. Increased expression of VEGF and its receptors in hypoxic areas, in association with interaction with cell membrane integrins including $\alpha_5\beta_3$, is one of the main causes of vessel leakiness [36]. Leaky plaque VV have been identified by ultrastructural visualization of defects between endothelial tight, gap and adherens junctions, and VEGF is known to affect junctional adhesion molecule expression, block gap junctional communication between adjacent endothelial cells and disrupt tight junctional communication through a src-dependent pathway [37].

Oxidized phospholipids such as 1-palmitoyl-2-arachidonoyl-*sn* -glycero-3-phosphorylcholine (Ox-PAPC), also prevalent in atherosclerotic lesions, can also up-regulate VEGF expression. In addition, they can regulate leukocyte-endothelial cell interactions and induce expression of inflammatory cytokines from local endothelial cells, monocytes and macrophages [38].

Pharmacological inhibition of VV angiogenesis inhibits plaque development and instability in vivo models of atherosclerosis

In vivo studies showed that blocking blood vessel formation can significantly reduce plaque size. Moulton *et al* , [50] demonstrated that two potent angiogenesis inhibitors, endostatin and TNP-470 were able to reduce neovessel formation and concomitant aortic plaque growth in apolipoprotein E (ApoE)-deficient mice. Recently, Stefanadis *et al* , [51], showed that treatment of New Zealand rabbits under atherogenic diet, with antibodies specific for VEGF, resulted in significant reduction in neovessel growth and neointimal thickness after four weeks. Similarly, Gossl *et al* , [52] showed that inhibition of VV neovascularization in high cholesterol diet treated domestic swine using thalidomide, significantly reduced neointimal plaque formation and intima-media thickness. The same authors also demonstrated that areas of the coronary vascular wall with low density of VV were most susceptible to microinflammation, hypoxia and oxidative stress making them most likely the starting points of early atherogenesis [53]. Similarly, Drinane et al [54] showed that treatment of LDLR(-/-) ApoB-48-deficient mice with an anti-angiogenic truncated form of plasminogen activator inhibitor (PAI) inhibited VV growth through a mechanism involving FGF-2 and resulted in reduced plaque development. Reduced blood flow to the end branches of the VV as seen in patients with vascular risk factors could lead to inflammation followed by increased permeability, LDL-lipoprotein up-take and macrophage phagocytosis leading to foam cell formation [55]. These results suggest that anti-angiogenic therapy may have beneficial effects in patients by modulating plaque vascularization. Whilst it is important to have normal healthy functional VV, prevention of their proliferation could represent the first stage of therapy against atherosclerotic plaque formation.

Conclusion

Current imaging methodologies may be used in conjunction with emerging therapies based on the use of angiogenesis-targeted nanoparticles which will allow both the identification and tracking of drugs and also prove to be more effective for the delivery of drugs to target sites. The preferential aim would be to prevent initial development of neointimal vascular sites by blocking adventitial blood vessel activation in patients with low grade plaques might represent 1) future imaging targets able to identify patients developing unstable plaque regions susceptible to rupture, and 2) prevent or slow down development of atheroma thereby improving treatment and survival rates of patients with a history of development of myocardial infarction or ischaemic stroke.

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References

1. Fishbein MC. The vulnerable and unstable atherosclerotic plaque. Cardiovasc Pathol. 2008.
2. Folkman J. Tumour angiogenesis: Therapeutic implications. N Engl J Med. 1971;285:1182-1186.
3. Slevin M, Kumar P, Gaffney J, Kumar S, Krupinski J. Can angiogenesis be exploited to improve stroke outcome? Mechanisms and therapeutic potencial. Clin Sci. 2006;111:171-183.
4. Chen CH, Walterscheid JP. Plaque angiogenesis versus compensatory arteriogenesis in atherosclerosis. Circ Res. 2006;99:787-789.
5. Krupinski J, Stroemer P, Slevin M, Marti E, Kumar P, Rubio F. Three-dimensional structure and survival of newly formed blood vessels after focal cerebral ischemia. Neuroreport. 2003;14:1171-1176.
6. Moreno PR, Purushothaman KR, Zias E, Sanj J, Fuster V. Neovascularization in human atherosclerosis. Curr Mol Med. 2006;6:457-477.
7. Moulton KS. Angiogenesis in atherosclerosis: gathering evidence beyond speculation. Curr Opin Lipidol. 2006;17:548-555.
8. Herrmann J, Lerman LO, Mukhopadhyay D, Napoli C, Lerman A. Angiogenesis in atherogenesis. Arterioscler Thromb Vasc Biol. 2006;26:1948-1957.
9. Piao M, Tokunaga O. Significant expression of endoglin (CD105), TGFβ-1 and TGFβR-2 in the atherosclerotic aorta: An immunohistological study. J Atheroscler Thromb. 2006;13:82-89.
10. Kumamoto M, Nakashima Y, Sueishi K. Intimal neovascularization in human coronary atherosclerosis: its origin and pathophysiological significance. Hum Pathol. 1995;26:450-456.
11. Juan-Babot JO, Martínez-González J, Berrozpe M, Badimon L. Neovascularization in human coronary arteries with lesions of different severity. Rev Esp Cardiol. 2003;56:978-986.
12. Virmani R, Kolodgie FD, Burke AP, Finn AV, Gold HK, Tulenko TN, Wrenn SP, Narula J. Atherosclerotic plaque progression and vulnerability to rupture: angiogenesis as a source of intraplaque haemorrhage. Arterioscler Thromb Vasc Biol. 2005;25:2054-2061.
13. McCarthy MJ, Loftus IM, Thompson MM, Jones L, London NJ, Bell PR, Naylor AR, Brindle N. Angiogenesis and the atherosclerotic carotid plaque: an association between symptomatology and plaque morphology. J Vasc Surg. 1999;30:261-268.
14. Khurana R, Simons M, Martin JF, Zachary IC. Role of angiogenesis in cardiovascular disease: a critical appraisal. Circulation. 2005;112:1813-1824.
15. O'Brien ER, Garvin MR, Dev R, Stewart DK, Hinohara T, Simpson JB, Schwartz SM. Angiogenesis in human coronary atherosclerotic plaques. Am J Pathol. 1994;145:883-894.
16. Khurana R, Zhuang Z, Bhardwaj S, Murakami M, De Muinck E, Yla-Herttuala S, Ferrara N, Martin JF, Zachary I, Simons M. Angiogenesis-dependent and independent phases of intimal hyperplasia. Circulation. 2004;110:2436-2443.
17. Raj T, Kanellakis P, Pomilio G, Jennings G, Bobik A, Agrotis A. Inhibition of fibroblast growth factor receptor signaling attenuates atherosclerosis in apoolipoprotein E-deficient mice. Arterioscler Thromb Vasc Biol. 2006;26:1845-1851.
18. Dunmore BJ, McCarthy MJ, Naylor AR, Brindle NP. Carotid plaque instability and ischaemic symptoms are linked to immaturity of microvessels within plaques. J Vasc Surg. 2007;45:155-159.
19. Moulton KS. Plaque angiogenesis and atherosclerosis. Curr Atheroscler Rep. 2001;3:225-233.
20. Stary HC, Chandler AB, Dinsmore RE, Fuster V, Glagov S, Insull W, Rosenfeld ME, Schwartz CJ, Wagner WD, Wissler RW. 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. 1995;15:1512-1531.
21. Mofidi R, Powell TI, Crotty T, Mehigan D, Macerlaine D, Keaveny TV. Angiogenesis in carotid atherosclerotic lesions is associated with timing of ischemic neurological events and presence of computed tomographic cerebral infarction in the ipsilateral cerebral hemisphere. Ann Vasc Surg. 2008;22:266-272.
22. Moulton KS, Vakili K, Zurakowski D, Soliman M, Butterfield C, Sylvin E, Lo KM, Gillies S, Javaherian K, Folkman J. Inhibition of plaque neovascularization reduces macrophage accumulation and progression of advanced atherosclerosis. Proc Natl Acad

Sci. 2003;100:4736-4741.

23. Kolodgie FD, Gold HK, Burke AP, Fowler DR, Kruth HS, Weber DK, Farb A, Guerrero LJ, Hayase M, Kutys R, Narula J, Finn AV, Virmani R. Intraplaque haemorrhage and progression of coronary atheroma. N Eng J Med. 2003;349:2316-2325.

24. Kolodgie FD, Burke AP, Nakazawa G, Cheng Q, Xu X, Virmani R. Free cholesterol in atherosclerotic plaques: where does it come from?. Curr Opin Lipidol. 2007;18:500-507.

25. Katsargyris A, Klonaris C, Bastounis E, Theocharis S. Toll-like receptor modulation: a novel therapeutic strategy in cardiovascular disease?. Expert Opin Ther Targets. 2008;12:1329-1346.

26. Frantz S, Vincent KA, Feron O, Kelly RA. Innate immunity and angiogenesis. Circ Res. 2005;96:15-26.

27. Hansson GK, Robertson AK, Söderberg-Nauclér C. Inflammation and atherosclerosis. Annu Rev Pathol. 2006;1:297-329.

28. Hayden MR, Tyagi SC. Vasa vasorum in plaque angiogenesis, metabolic syndrome, type 2 diabetes mellitus, and atheroscleropathy: a malignant transformation. Cardiovasc Diabetol. 2004;4:1-.

29. Zhao Q, Egashira K, Hiasa K, Ishibashi M, Inoue S, Ohtani K, Tan C, Shibuya M, Takeshita A, Sunagawa K. Essential role of vascular endothelial growth factor and Flt-1 signals in neointimal formation after peri-adventitial injury. Arterioscler Thromb Vasc Biol. 2004;24:2284-2289.

30. Langheinrich AC, Sedding DG, Kampschulte M, Moritz R, Wilhelm J, Haberbosch WG, Ritman EL, Bohle RM. 3-Deazaadenosine inhibits vasa vasorum neovascularization in aortas of ApoE (-/-)/LDL(-/-) double knockout mice. Atherosclerosis. 2008;202:103-110.

31. Moreno PR, Purushothaman KR, Fuster V, Echeverri D, Truszczynska H, Sharma SK, Badimon JJ, O'Connor WN. Plaque neovascularization is increased in ruptured atherosclerotic lesions of human aorta: implications for plaque vulnerability. Circulation. 2004;110:2032-2038.

32. Williams JK, Armstrong ML, Heistad DD. Vasa vasorum in atherosclerotic coronary arteries: response to vasoactive stimuli and regression of atherosclerosis. Circ Res. 1988;62:515-523.

33. Trape J, Morales C, Molina R, Filella X, Marcos JM, Salinas R, Franquesa J. Vascular endothelial growth factor serum concentration in hypercholesterolemic patients. Scand J Clin Invest. 2006;66:261-267.

34. Garbin U, Fratta Pasini A, Stranieri C, Manfro S, Mozzini C, Boccioletti V, Pasini A, Cominacini M, Evangelista S, Cominacini L. Effects of nebivolol on endothelial gene expression during oxidative stress in human umbilical vein endothelial cells. Mediators Inflamm. 2008;2008:367590-.

35. Ziello JE, Jovin IO, Huang Y. Hypoxia-inducible factor (HIF)-1 regulatory pathway and its potential for therapeutic intervention in malignancy and ischaemia. J Biol Med. 2007;80:51-60.

36. Weis SM, Cheresh DA. Pathophysiological consequences of VEGF-induced vascular permeability. Nature. 2005;437:497-504.

37. Suarez S, Ballmer-Hofer K. VEGF transiently disrupts gap junctional communication in endothelial cells. J Cell Sci. 2001;114:1229-1235.

38. Khan M, Pelengaris S, Cooper M, Smith C, Evan G, Betteridge J. Oxidised lipoproteins may promote inflammation through the selective delay of engulfment but not binding of apoptotic cells by macrophages. Atherosclerosis. 2003;171:21-29.

39. Moulton KS, Olsen BR, Sonn S, Fukai N, Zurakowski D, Zeng X. Loss of collagen XVIII enhances neovascularization and vascular permeability in atherosclerosis. Circulation. 2004;110:1330-1336.

40. Xu X, Lu H, Lin H, Li X, Ni M, Sun H, Li C, Jiang H, Li F, Zhang M, Zhao Y, Zhang Y. Aortic adventitial angiogenesis and lymphangiogenesis promote intimal inflammation and hyperplasia. Cardiovasc Pathol. 2008.

41. Brindle NP, Saharinen P, Alitalo K. Signalling and functions of angopoietin-1 in vascular protection. Circ Res. 2006;98:1014-1023.

42. Post S, Peeters W, Busser E, Lamers D, Sluijter JP, Goumans MJ, de Weger RA, Moll FL, Doevendans PA, Pasterkamp G, Vink A. Balance between Angiopoietin-1 and Angiopoietin-2 is in favour of Angiopoietin-2 in atherosclerotic plaques with high microvessel density. J Vasc Res. 2008;45:244-250.

43. Peters KG, Coogan A, Berry D, Marks J, Iglehart JD, Kontos CD, Rao P, Sankar S, Trogan E. Expression of Tie2/Tek in breast tumour vasculature provides a new marker for evaluation of tumour angiogenesis. Br J Cancer. 1998;77:51-56.

44. Chen JX, Stinnett A. Ang-1 gene therapy inhibits HIF-1 (alpha)-prolyl-4-hydroxylase-2, stabilizes HIF-1 (alpha) expression and normalizes immature vasculature in db/db mice. Diabetes. 2008;57:3335-3343.

45. Pilarczyk K, Sattler KJE, Galili O, Versari D, Olson ML, Meyer FB, Zhu XY, Lerman LO, Lerman A. Placenta growth factor expression in human atherosclerotic carotid plaques is related to plaque destabilization. Atherosclerosis. 2008;196:333-340.

46. Leroyer AS, Rautou PE, Silvestre JS, Castier Y, Leseche G, Devue C, Duriez M, Brandes RP, Lutgens E, Tedgui A, Boulanger CM. CD40 ligand+ microparticles from human atherosclerotic plaques

stimulate endothelial proliferation and angiogenesis a potential mechanism for intraplaque neovascularization. J Am Coll Cardiol. 2008;52:1302-1311.

47. Roth JJ, Gahtan V, Brown JL, Gerhard C, Swami VK, Rothman VL, Tulenko TN, Tuszynski GP. Thrombospondin-1 is elevated with both intimal hyperplasia and hypercholesterolemia. J Surg Res. 1998;74:11-16.

48. Sachais BS, Turrentine T, Dawicki McKenna JM, Rux AH, Rader D, Kowalska MA. Elimination of platelet factor 4 (PF4) from platelets reduces atherosclerosis in C57Bl/6 and apoE-/- mice. Thromb Haemost. 2007;98:1108-1113.

49. Rubina K, Kalinina N, Potekhina A, Efimenko A, Semina E, Poliakov A, Wilkinson DG, Parfyonova Y, Tkachuk V. T-cadherin suppresses angiogenesis in vivo by inhibiting migration of endothelial cells. Angiogenesis. 2007;10:183-195.

50. Moulton KS, Heller E, Konerding MA, Flynn E, Palinski W, Folkman J. Angiogenesis inhibitors endostatin or TNP-470 reduce intimal neovascularization and plaque growth in apolipoprotein E-deficient mice. Circulation. 1999;99:1726-1732.

51. Stefanadis C, Toutouzas K, Stefanadi E, Lazaris A, Patsouris E, Kipshidze N. Inhibition of plaque neovascularization and intimal hyperplasia by specific targeting vascular endothelial growth factor with bevacizumab-eluting stent: an experimental study. Atherosclerosis. 2007;195:269-276.

52. Gossl M, Herrmann J, Tang H, Versari D, Galili O, Mannheim D, Rajkumar SV, Lerman LO, Lerman A. Prevention of vasa vasorum neovascularization attenuates early neointima formation in experimental hypercholesterolemia. Basic Res Cardiol. 2009.

53. Gossl M, Versari D, Lerman LO, Chade AR, Beighley PE, Erbel R, Ritman EL. Low vasa vasorum densities correlate with inflammation and subintimal thickening: Potential role in location-Determination of atherogenesis. Atherosclerosis. 2009.

54. Drinane M, Mollmark J, Zagorchev L, Moodie K, Sun B, Hall A, Shipman S, Morganelli P, Simons M, Mulligan-Kehoe MJ. The antiangiogenic activity of rPAI-1 (23) inhibits vasa vasorum and growth of atherosclerotic plaque. Circ Res. 2009;104:337-345.

55. Jarvilehto M, Tuohimaa P. Vasa vasorum hypoxia: initiation of atherosclerosis. Med Hypotheses. 2009;73:40-41.

56. Roy S, Patel D, Khanna S, Gordillo GM, Biswas S, Friedman A, Sen CK. Transcriptome-wide analysis of blood vesselslaser captured from human skin and chronic wound-edge tissue. Proc Natl Acad Sci USA. 2007;104:14472-14477.

57. Hellstrom M, Phng LK, Hofmann JJ, Wallgard E, Coultas L, Lindblom P, Alva J, Nilsson AK, Karlsson L, Gaiano N, Yoon K, Rossant J, Iruela-Arispe ML, Kalen M, Gerhardt H, Betsholtz C. Dll4 signalling through Notch1 regulates formation of tip cells during angiogenesis. Nature. 2007;445:776-780.

58. George J, Afek A, Abashidze A, Shmilovich H, Deutsch V, Kopolovich J, Miller H, Keren G. Transfer of endothelial progenitor and bone marrow cells influences atherosclerotic plaque size and composition I apolipoprotein E knockout mice. ATVB. 2005;25:2636-2641.

59. Magnoni M, Coli S, Marrocco-Trischitta MM, Melisurgo G, De Dominicis D, Cianflone D, Chiesa R, Feinstein SB, Maseri A. Contrast-enhanced ultrasound imaging of periadventitial vasa vasorum in human carotid arteries. Eur J Echocardiogr. 2009;10:260-264.

60. Granada JF, Feinstein SB. Imaging of the vasa vasorum. Nat Clin Pract Cardiovasc Med. 2008;5:18-25.

61. Xu Q. The impact of progenitor cells in atherosclerosis. Nat Clin Pract Cardiovasc Med. 2006;3:94-101.

62. Siow RC, Churchman AT. Adventitial growth factor signalling and vascular remodelling: potential of perivascular gene transfer from the outside-in. Cardiovasc Res. 2007;75:659-668.

63. Wickline SA, Neubauer AM, Winter PM, Caruthers SD, Lanza GM. Molecular imaging and therapy of atherosclerosis with targeted nanoparticles. J Mag Res Imag. 2007;25:667-680.

64. Kelly KA, Allport JR, Tsourkas A, Shinde-Patil VR, Josephson L, Weissleder R. Detection of vascular adhesion molecule-1 expression using a novel multimodal nanoparticle. Circ Res. 2005;96:327-336.

65. Shah F, Balan P, Weinberg M, Reddy V, Neems R, Feinstein M, Dainauskas J, Meyer P, Goldin M, Feinstein SB. Contrast-enhanced ultrasound imaging of atherosclerotic carotid plaque neovascularization: a new surrogate marker of atherosclerosis. Vasc Med. 2007;12:291-297.

66. Jarvelainen H, Sainio A, Koulu M, Wight TN, Penttinen R. Extracellular matrix molecules: Potential targets in pharmacology. Pharmacol Rev. 2009;61:198-223.

67. Jain RK, Finn AV, Kolodgie FD, Gold HK, Virmani R. Anti-angiogenic therapy for normalization of atherosclerotic plaque vasculature: a potential strategy for plaque stabilization. Nat Clin Prac Cardiovasc Med. 2007;4:491-502.

68. Eyries M, Siegfried G, Ciumas M, Montagne K, Agrapart M, Lebrin F, Soubrier F. Hypoxia-induced apelin expression regulates endothelial cell proliferation and regenerative angiogenesis. Circ Res. 2008;103:432-440.

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