POST-PROCEDURAL INTIMAL HYPERPLASIA

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REZUMAT

Angioplastia percutană reprezintă tratamentul de primă linie pentru stenozele aterosclerotice de la nivelul membrelor inferioare, al arterelor coronare, arterelor renale. Angioplastia percutană constă din: dilatarea vasului, compresia plăcii și fisurarea componentelor sale. Ruptura, fractura plăcii de aterom are o extindere variabilă și un singur scop: îmbunătățirea diametrului vascular și a fluxului sanguin. Modificările rezultate din vindecarea acestei lezări constau dintr-o remodelare urmată fie de lărgirea lumenului vascuar, fie de scăderea lui (restenoză). Au fost examinate 30 de segmente coronariene de la pacienți care au suportat angioplastie percutană cu stentare și 30 de segmente coronariene de la pacienți coronarieni care nu au beneficiat de indicația stentării. Materialul a fost prelucrat de rutină și examinat histopatologic în microscopie optică. Examenul clasic pentru microscopul optic a inclus: 3-4 blocuri din fiecare caz, care au fost secționate la 5 microni și au fost colorate hematoxilină-eozină, elastic (orceină) și tricrom masson. Examenul remodelării vasculare post lezionale s-a efectuat comparativ la nivelul segmentelor arteriale cu plăci aterosclerotice care au avut evoluția lor naturală. La imunocolorare s-au folosit secțiuni ale fragmentelor de vase descrise, incluse în parafină, după fixare in formol. Studii ale restenozei de stent au demonstrat că precoce, elementele implicate în răspunsul post lezional, după injuria mecanică directă (aplicarea de stent) sunt celulele musculare netede, deendotelizarea și formarea de tromb. În timp, celulele musculare netede proliferează și migrează, depun matricea extracelulară. Legătura dintre injuria vasculară și creșterea neointimală se realizează prin intermediul inflamației.

Cuvinte cheie: angioplastie percutană, stent, segment coronar, restenoză

ABSTRACT

Percutaneous angiogplasty represents a first line therapy for atherosclerotic obstructions at the level of the lower extremities, coronary arteries and renal arteries. Percutaneous angioplasty consists of the dilation of the vessel, compression of the plaque and fissure of its components. The rupture the atheroma plaque varies in extension and is performed with one aim: to improve the vessel diameter and blood flow. The changes resulting from the healing of this lesion consisted in a re-modellation followed either by the enlargement of the vascular lumen, or by its narrowing (re-obstruction). The study was performed on 30 coronary segments from patients with percutaneous angioplasty with stent placement, and 30 coronary segments in which stent placement was not indicated. The samples were prepared by routine methods and examined histopathologically by light microscopy. The classical microscopic examination included: 3-4 blocks for each case, sectioned at 5 microns, stained by hematoxylin eosin, elastic (orcein) and trichrome masson. The examination of vascular post-lesion remodelling was performed comparatively at the level of the arterial segments with atherosclerotic plaques undergoing a natural evolution. For immunohistochemistry sections of the study samples were used, embedded in paraffin after fixation in formaldehyde 10%. The investigation of the stent re-stenosis have shown that at an early stage the elements involved in the post-lesion response, after direct mechanical injury (stent placement), are the smooth muscle cells, endothelium loss and thrombus formation. In time the smooth muscle cells proliferate and migrate, depositing the cellular matrix. The relationship between the vascular injury and the neointimal growth is achieved through inflammation.

Key Words: percutaneous angioplasty, stent, coronary segments, restenosis

INTRODUCTION

In the last years literature has reflected the need to define the cell types participating in vascular remodeling. The identification of smooth muscle cells in intimal hyperplasia as well as in the atheroma plaque draws the attention to the source of cells that form the neointima. The vascular changes following angioplasty, the use of venous segments for arterial replacement and, last but not least, the vascular changes following heart transplantation represent topics with major practical implications. Atherosclerotic lesions consist of areas of musculo-fibrous proliferation, with accumulation of foamy cells, lipid-laden macrophages. The presence of the inflammatory infiltrate at the intima-media junction or in the plaque is related to activation of the plaque and appearance of acute coronary syndromes. Interventional cardiology brings to the forefront the modern and efficient methods of treatment of coronary disease.

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MATERIAL AND METHODS

The study was carried out on 30 coronary segments collected from patients submitted to percutaneous angioplasty with stent placement, and 30 coronary segments from patients in whom a stent was not placed. The patient group included seven men and three women, mean age 52.7 years. The group with stent placement included eight men and two women, mean age 54.3 years. Epicardial coronary segments from 10 cases of coronary death, without stenting, were also examined. The samples underwent paraffin embedding according to routine protocols; histopathological examination was performed by light microscopy. The classical examination included 3-4 blocks for each patient, sectioned at 5 microns; the sections were stained with: hematoxylin eosin, elastic (orcein), and trichrome masson. The study of post-injury vascular remodeling was performed comparatively at the level of the arterial segments and in atherosclerotic plaques with natural evolution.

For immunohistochemistry sections were processed thermally for 15 minutes in citrate buffer at pH 6. Primary antibodies were applied to the 1:100 dilution vimentin, 1:100 alpha actin for smoothe muscle; 1:50 for CD_{68}, 1: 50 desmin, and 1:200 CD_{20}. For technical visualization the biotin streptavidin system was used (LSAB kit). The chromogenic substrate (for peroxidase) was diaminobenzidine (DAB). For this reason, positive cells were brown, contra-staining being performed with aqueous hematoxylin eosin, which made the background appear blue.

RESULTS

The American Heart Association, through its Committee on Vascular Lesions, published a definition of coronary atherosclerotic lesions, which includes: initial lesions, type II lesions (lipid-laden cells), intermediate lesions (type III); atheroma are considered type IV lesions, type V is represented by fibrous plaques, while type VI are complicated lesions (erosion, hemorrhage, thrombus).[1]

The atherosclerotic lesions studied followed this classification.

Distribution and type of coronary lesions are presented in Tables 1 and 2.

The plaque may undergo bleeding, calcification, thrombosis, complications evidenced in the bioptic sample. In our case the atheroma plaques that caused myocardial infarction with our without stent were types V and VI.

Table 1. Distribution of coronary lesions in patients with stent placement.

Maximal coronary stenosis	No.	40%	> 80%
- RC	7	2	5
- LCCT	2	-	2
- LAD	11	2	9
- CX	8	1	7
Stent location			
- RC	4		
- CX	6		
- LAD	4		

RC - right coronary, LCCT - left coronary common trunk, LAD - left anterior descending coronary, CX - circumflex coronary, 40% - non-significant coronary obstruction, > 80% - severe coronary obstruction.

Table 2. Distribution of coronary lesions in patients without stent placement.

Maximal coronary stenosis	No.	40%	> 80%
- RC	9	1	8
- LCCT	2	-	2
- LAD	8	1	7
- CX	10	8	2
Stent location			
- RC	3		
- CX	5		
- LCCT	2		

RC - right coronary, LCCT - left coronary common trunk, LAD - left anterior descending coronary, CX - circumflex coronary, 40% - non-significant coronary obstruction, > 80% - severe coronary obstruction.

The histological examination of atheroma plaques evidenced chronic adventitial inflammatory infiltrations, did not point to vasculitis and had T lymphocytes in their composition. In our study we have noticed the presence of an medioadventitial and periadventitial inflammatory infiltrate rich in B cells in the arterial segments that had had a stent for longer than a month. In a stent placed three years before, without thrombosis, trans-stent bridges were evidenced, similar to those noticed in the case of vascular prostheses. (Fig. 1) There were fenotype CD68 positive cells in these bridges. (Figs. 2-5) Adventitial inflammation was also present.

DISCUSSION

Intimal hyperplasia, neointima, are entities related to modern procedures: percutaneous angioplasty, coronary

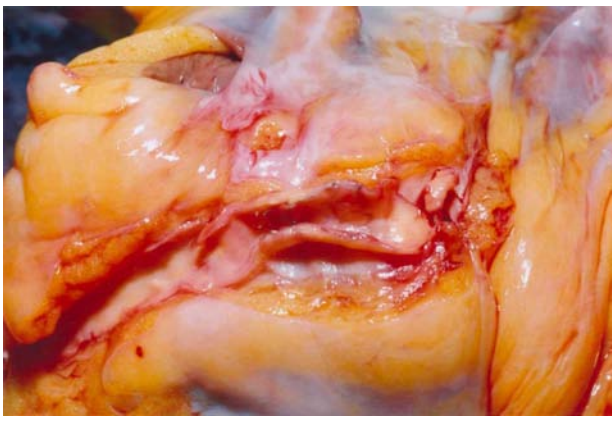

Figure 1. Stent in coronary artery segment after 3 years.

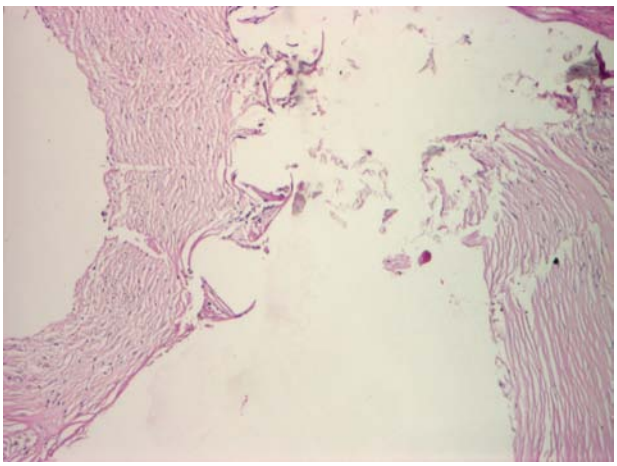

Figure 2. Neointimal fibrosis (stent 3 years after stenting). HE x4.

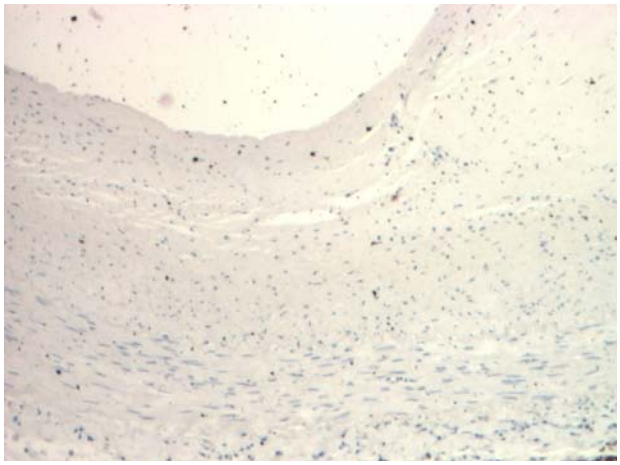

Figure 3. The adventitia of the stented coronary artery. Periadventitial inflammatory infiltrate rich in B cells CD20. x4

bypass, but also to atherosclerosis itself and the alterations found in the post-transplantation heart. Exposure of the vessels to excessive hemodynamic stress (hypertension), the toxic effects of blood components (atherogeneous lipids), local release of cytokines (post-angioplasty), all require the presence of mechanisms that would counter the adverse reactions in order to maintain the balance and the integrity of the vascular wall. These reactions of restorative nature determine the narrowing of the vascular lumen.[2-9]

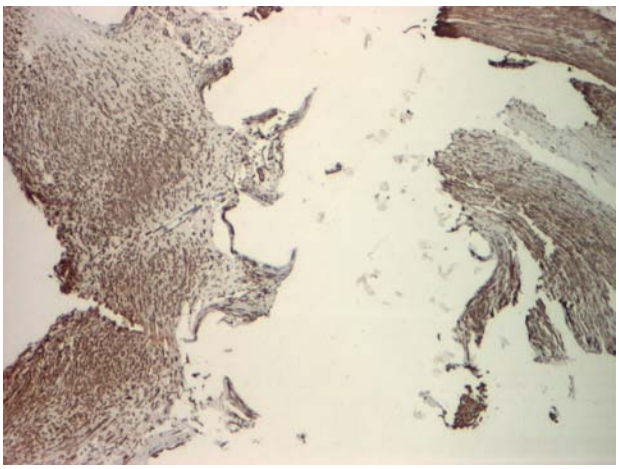

Figure 4. Alpha smooth muscle actin at intimal and at medial level. x4.

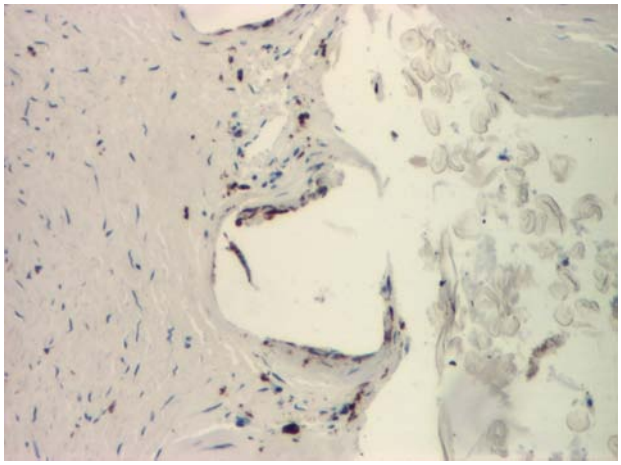

Figure 5. Trans stent bridges rich in CD68 cells. x10.

Veins do not develop atherosclerosis in conditions of their reduced pressure; placing venous segments in high pressure conditions is followed by the rapid onset of atherosclerotic lesions.[10] The use of autologous venous segments for revascularization procedures is accompanied by the structural remodeling of the thickness of the whole segment. Besides the mechanical forces, intimal hyperplasia of a vein placed in an arterial environment is further favored by adhesion molecules, cytokines, reactive oxygen species and matrix proteins. Subsequent intimal hyperplasia is attributed to phenotype changes of the media smooth muscle cells and also to the contribution of circulating precursor cells derived from a medullar level. In experimental conditions, in a venous graft model transplanted for the study of intimal thickening, both donor and receiver cells contributed to the accumulation of smooth muscle cells in the intima.

Percutaneous angioplasty represents a first line treatment for atherosclerotic obstruction at the level of the lower extremities, coronary arteries and renal arteries. It consists of: dilation of the vessel, compression of the plaque and fissure of its components with the one aim to improve the vascular

diameter and blood flow. The changes resulted from the healing of this wound consist of a remodeling followed by the vascular lumen enlargement or by its narrowing (re-obstruction). Re-stenosis occurs mainly when the fissure affects the muscular layer of the coronary at deeper levels.[11-17] The morphological basis of chronic re-stenosis is the hyperplasia of smooth muscle cells and fibrosis. The accepted theory of the re-stenosis mechanism incriminates the endothelial and medial response (smooth muscle cells response) to mechanical injury. It is possible that the same mechanism that initiates the atheromatous plaque fissure also triggers the vessel re-obstruction.[12,18-24] In any case, post-procedural fibro-cellular proliferation may be followed by the reduction of the vascular lumen by the migration and proliferation of smooth muscle cells from the coronary media. Coronary stenting had as first objective the minimization of re-stenosis, At present a stent placement represents a routine procedure performed by the interventional cardiologist. Percutaneous angioplasty with stent placement has become the method of choice in the treatment of coronary disease. If in 1995 FDA (Food and Drug Administration) accepted two stent types, nowadays there are at least 16 models from nine different manufacturers.[13] The use of stents has reduced re-obstruction as compared to angioplasty without stent placement, but stent-induced re-stenosis remains a serious problem. Anti-thrombotic therapy was not very effective in minimizing re-obstruction, therefore the stent manufacturers developed stents impregnated with drugs. Since 2004, at least 35 types of drug-eluting stents have been launched on the market. Drugs such as sirolimus act locally as neointimal inhibitor, reducing smooth muscle cell proliferation and inflammation. Clinical studies of the drug-eluting stents reported promising results regarding reobstruction and consequently they were introduced into current practice.[14-17,25-28]

Studies of stent re-stenosis have shown that, at an early stage, the elements involved in the post-injury response, following direct mechanical injury (stent placement) are the smooth muscle cells, endothelium loss and thrombus formation. In the process, smooth muscle cells proliferate and migrate, depositing the extra-cellular matrix. The link between vascular injury and neointimal growth is achieved through inflammation. The extensive use of stents alters the post-lesional vascular response by producing prolonged and intense inflammation.

A common feature of atherosclerotic lesions is their development on segments submitted to high biomechanical stress.

Coronary stenting followed by lesions of the coronary media or the penetration of the stent into a lipid-laden plaque (in the lipid middle of the plaque) is also followed by an increase of the inflammation at the level of the arterial wall and more intense neointimal thickening.[17,28-32]

The rate of re-obstruction is higher in diabetic patients, in stent-induced obstructions and in lesions placed at the bifurcation of vessels.[14]

Literature is rich in studies regarding the implication of inflammation in atherosclerosis and thrombosis, the onset of lesions and alteration of the vascular wall balance. Intimal thickening following mechanical injury plays a decisive role after stent placement, because stenting does not allow the vascoconstriction of the remodeled vessel. A number of experimental studies have explained the changes evidenced after platelet and fibrin deposits at the lesion level and by the activation of inflammation genes. In this scenario, the next sequence is the increase of leukocytes circulation at the level of the injured vascular wall and neointimal formation. Some authors place the monocytic macrophages as first inflammatory cells, others, based on balloon angioplasty models, involve neutrophils. The origin of smooth muscle cells of the neointima may be the media of the stented coronary or the adventitial myofibroblasts. The latter may be attracted to the lesion by chemotactic or mitogenic substances released by the arterial injury and by the inflammatory cells. More recently circulating progenitors have been evidenced, derived from the medulla or other organs located at the level of the vascular lesion, transforming into smooth muscle cells and thus contributing to the neointima formation. These theories still await confirmation.[15-17,26-28,31]

The investigation of the cellular events in an injured vascular wall has demonstrated the presence of inflammatory elements within the first hours and days after the injury and the persistence of inflammation for days and months in case of stent placement. Inhibition of the accumulation of inflammatory cells reduces the neointima. P selectin is expressed by the activated platelets that adhere to the level of the post-lesional endothelial denudation and also to the activated and regenerative endothelium a few weeks after injury. The inhibition of the P selectin effect and the reduction of the intimal thickening in a model of carotid lesion in mice support the role of this molecule in post-lesion remodeling. Unfortunately the morphological data for human coronary segments are scarce, additional clinical trials being required. The presence of inflammation

markers in man may be associated with an increased risk of stent re-stenosis. The final aim of stents treated with anti-proliferative and antiinflammatory drugs is the reduction of the neointima.[14,15]

In atherosclerosis, intimal and adventitial neovascularization is increased, which represents other possible gateways for the inflammatory cells. For this reason, the mechanism by which the recruitment of inflammatory cells through the P selectin can be blocked remains unclear.

The response to vascular injury presents regional differences between arteries and between arteries and veins. The coronary arteries differ from the aorta and the peripheral arteries by embryological evolution (medial smooth muscle cells and adventitial fibroblasts originate from common pro-epicardial precursors), postnatal particular features (smooth muscle cells in the coronary media present features that reflect a slow growth and a well differentiated phenotype, protective mechanisms against obstructive lesions), homeostatic features of the media (marked expression of superoxide dismutase and of tissular factors inhibiting matriceal metalloproteins, which reduces oxidative stress and cell migration). All of these support the differences existing between the arterial regions regarding restorative processes. The much more aggressive venous lesions from the point of view of intimal hyperplasia are explained by a number of factors: anatomical (poorly defined lamina elastica), physiological (low levels of nitric oxide and prostacyclin, increased sensitivity to vasoconstriction), and hemodynamic (compliance, turbulence, flow stress).[4-6,31]

CONCLUSIONS

Smooth muscle cells are directly involved in post-lesion intimal hyperplasia. The knowledge of the mechanisms of intimal thickening provides information on the mechanisms of atherosclerosis. Venous post-lesion response is more extensive than the arterial one, due to structural, hemodynamic and functional causes. Coronary stenting represents a modern technique used for its benefits in the treatment of patients with coronary disease or atherosclerosis in other areas of the body. The results obtained raise a number of questions that need to be answered by further investigations. The presence of a stent in a vascular segment is associated with an inflammatory response, related to the thickness of the neointima. Drug-treated stents aiming at reducing local inflammation and neo-angiogenesis could minimize re-stenosis.[17]

REFERENCES

1. Stary HC, Chandler AB, Dinsmore RE, et al. 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 Atherosclerosis. Circulation 1995;92:1355-74.
2. Mayr M, Hu Y, Hainaut P, et al. Mecanical stress induced DNA damage and rac-p38 MAPK signal pathways mediate p 53 - dependent apoptosis in vascular smooth muscle cells. FASEB J 2002;16:1423-5.
3. Xu Q. Mouse models to ateriosclerosis: from arterial injuries to vascular grafts. Am J Pathol 2004;165:1-10.
4. Shi Y, O`Brien J, Mannion J, et al. Remodeling of autologous safenous vein grafts. The role of perivascular miofibroblasts. Circulation 1997;95:2684-93.
5. Baklanov DV, Peters KG, Seidel AL, et al. Neovascularisation in intimal hyperplasia is associated with vein graft failure after coronary artery by pass surgery. Vascular Medicine 2002;8:163-7.
6. Bonatti J, Oberhuber A, Schachner T, et al. REVIEW: Neointimal hyperplasia in coronary vein grafts- pathophysiology and prevention of significant clinical problem. Heart Surg Forum 2003;1:158-63.
7. Campos HA, Zhoa Y, Pollman M, et al. DNA microarray profiling to identify angiotensin - responsive genes in vascular smooth muscle cells. Circul Research 2003;92:111-21.
8. Davies JM, Mann JM. Acquired diseases of the heart. In Symmers WStC (Ed.) Systemic pathology. New York: Churchill Livingstone, 1995, p. 60-61.
9. Gimbrone MA. Vascular endothelium in health and disease. In Haber E (Ed.) Molecular cardiovascular medicine. New York: Scientific American Inc, 1995, p. 49-63.
10. Lentz S. Thrombosis of vein grafts. Circul Research 2003;92:12-27.
11. Waller B. Contemporary issues in cardiovascular pathology. Philadelphia: Davis, 1998, p. 187-217.
12. Topol E. Textbook of interventional cardiology. Philadelphia: Saunders, 1995, p. 395-427.
13. Butany J, Carmichael SW, Leong SV, et al. Coronary artery stents: identification and evaluation. J Clin Pathol 2005;58:795-804.
14. O Neill W, Martin L. Drug eluting stents. Circulation 2003;107:3008-13.
15. Shah PK. Inflammation, neointimal hyperplasia and restenosis. Circulation 2003;107:2175-81.
16. Welt GPF, Rogers C. Inflammation and restenosis in the stent era. Arterioscl Thromb Vasc Biol 2002;22:1769-77.
17. Farb A, Deena K, Weber MS. Morphological predictors of restenosis after coronary stenting in humans. Circulation 2002;105:2974-87.
18. Weber C. Platelets and chemokines in atherosclerosis. Circulation Res 2005;96:612-25.
19. Martinez Gonzales J, Rius J, Castello A, et al. Neurom derived orphan receptor-1 (NOR-1) modulates vascular smooth muscle cell proliferation. Circulation Res 2003;92:96-111.
20. Nagai R. Transcriptional regulation of smooth muscle phenotypic modulation. Proceedings of the XXIII International Congress of the International Academy of Pathology, Nagoya, 2000, p. 359-61.
21. Ryan JM, Eming LL, Hicks GW, et al. Localization of lipoprotein A in a mockey model of rapid neointimal growth. Ateriosclerosis Thromb Vasc Biol 1997;17:181-7.
22. Rose A. Diseases of veins. In Silver M (Ed.) Cardiovascular pathology. New York: Churchill Livingstone, 1991, p. 210-8.
23. Ross R. Arteriosclerosis: an overview. In Haber E (Ed.) Molecular cardiovascular medicine. New York: Scientific American, 1995, p. 11-31.
24. Virmani R, Atkinson BJ, Forman BM. Aortocoronary bypass grafts and extracardiac conduits. In Silver M (Ed.) Cardiovascular pathology. New York: Churchill Livingstone, 1991, p. 1607-49.
25. Ueda M. The pivotal role of the neointima in post PTCA and post stent restenosis Pathology of restenosis after angioplasty. Proceedings of the XXIII International Congress of the International Academy of Pathology, Nagoya, 2000, p. 362-4.

26. Yonemitsu Y, Sueishi K. Gene therapy for atherosclerosis and restenosis: application of recombinant Sedai virus as a novel gene transfer agent. Proceedings of the XXIII International Congress of the International Academy of Pathology, Nagoya, 2000, p. 513-8.
27. Hu Y, Zhang Z, Torsney E, et al. Abundant progenitor cells in the adventitia contibute to atherosclerosis of vein grafts in Apo E deficient mice. J Clin Invest 2004;113:1258-65.
28. Hoofnagle M, Wamhoff BR, Owens G. Lost of transdifferentiation. J Clin Invest 2004;113:1249-51.
29. Raucher FM, Goldschmidt Clermont PJ, Davis BH. Aging, progenitor cells exhaustion and atherosclerosis. Circulation 2003;108:457-63.
30. Rey F, Patrick JP. The reactive adventitia. Fibroblast oxidase in vascular function. Aterioscler Thromb Vasc Biol 2002;22:1962-71.
31. Li G, Chen SJ, Oparil S., et al. Direct in vivo evidence demonstrating neointimal migration of adventitial fibroblasts after ballon injury of rat carotid arteries. Circulation 2000;101:1362-5.
32. Tanaka K, Sata M, Nagai R. Diverse contribution of bone marrow cells in neointimal hyperplasia after mechanical vascular injuries. Circ Res 2003;93:783-90.