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Rearrangements of actin cytoskeleton in atherosclerotic plaque development (experimental study)

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Motivation and Aim: Atherosclerosis of the blood vessels of different localization is one of the main causes of mortality in the industrial countries [1]. The detailed conception of pathogenesis of this disease is very important for development of effective methods of prophylaxis and treatment. The cellular mechanisms of atherosclerosis development, particularly patterns of foam cells formation and behavior on the blood vessel inner surface during atherogenesis is poorly studied. The aim of study: to characterize to characterize the peculiarities of 3D-structure of actin cytoskeleton in atherosclerotic plaque morphogenesis.

Methods and Algorithms: Atherosclerosis was induced on "Chinchilla" rabbits with high-cholesterol diet (0.35 g of cholesterol per 1 kg of animal weight) during 3 months. Animals of control group was fed with standard diet. Rabbit peritoneal macrophages [2] was used for comparative control in foam cells cytochemical peculiarities studying. Aorta fragments and peritoneal macrophages were fixed with 4 % paraformaldehyde, permeabelized with 0.2 % Triton-X100, F-actin was stained with Alexa Fluor 488 Phalloidin, lipid droplets – Nile red, nuclei – Hoechst 33342. Slides were analyzed with laser confocal microscope LSM 710 (Zeiss Germany).

Results: Study of structural and functional peculiarities of peritoneal macrophages showed that in cell suspension they are spherical and they have dense submembrane actin cortex and some of them contains rare small lipid droplets in cytoplasm. There are not any blood cells adhere to endothelium on the aorta inner surface of the rabbit from control group. After 2 weeks of high-cholesterol diet were registered macrophages on the surface of aorta. These cells have spindle shape, contain multiple thin axially oriented actin microfilaments and practically do not carry lipid droplets. In the whole, macrophages on the aorta surface oriented parallel to the blood flow. Front edge of some cells trapezoidal widened and have filopodia, that is suggest about migratory activity. Basically, macrophages does not contact with one another and this cells forms clusters in the places of arteria brunching from aorta. On the late stages of atherogenic diet in these locations forms atherosclerotic plaques. The analysis of rabbit aorta after 3 months of high-cholesterol diet, allowed reconstruction of the subsequent stages of atherogenesis. According to these data, non-contacting with one another macrophages begin deposit lipids, gradually transform to foam cells. Further plaque morphogenesis occurs through growth of quantity and volume of lipid droplets of existing foam cells, recruiting new macrophages, forming lateral contacts between neighbor cells with

building of monolayer, and later – multilayer structures. 3D Analysis of multilayer atherosclerotic plaques shows high morphological diversity of their cells: spheroidal, ellipsoidal, spindle, and pyramidal shaped cells. It can be assumed that cells of different form have different role and various type of activity in the tissue. The most of the cells contain thick actin cables oriented parallel to each other along the entire length of the cells. Among those cells are located the cells without thick actin cables. The cells with thick actin cables contacts with one another to form integral rigid actin framework of atherosclerotic plaque. Obviously, this is an adaptation of cell population of atherosclerotic plaque for preservation of integrity of structure in the conditions of shear stress generated with hydrodynamic forces of blood flow. It is possible that disruption structure of integral actin framework will be promote to atherosclerotic plaque destabilization and rupture, that is the main cause of thrombus formation.

Conclusion: Thus, in the beginning of atherogenesis the areas of monocyte/macrophage adhesion on the inner surface of aorta predetermines the shape of developing atherosclerotic plaque. Cells, composing the plaque are variable in shape, lipid droplets content and actin cytoskeleton structure. Nevertheless, the population of those cells have rigid integral actin framework, and, apparently, is a single morpho-functional complex. The studying of the patterns of morphogenesis and behavior of those cellular complexes will be promote understanding the mechanisms of the appearance of vulnerable plaques and the development of technologies for the diagnosis, prevention and treatment of complications of atherosclerosis.

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