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In Vitro Culture of Endothelial Cell and Smooth Muscle Cell for Studying Vascular Diseases

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－Abstract－

Endothelial cells play a key role in pathological processes such as cancer cell metastasis, atherosclerosis, and diabetic retinopathy. Vascular smooth muscle cells directly involve in the formation of atheroma in atherosclerosis. Some kinds of the endothelial cells are simply harvested from the umbilical veins, the tunica intima of aortic walls, the retina using various enzymes solutions. Those purely isolated cells provide a powerful tool in vitro studies of the endothelial cell related diseases. In this context, the cultured smooth muscle cells after the isolation from the tunica media of aortic walls are also used for elucidating the pathogenesis of atherosclerosis. Here, I briefly introduce articles that include the isolation of human umbilical vein endothelial cells (HUVEC), aortic endothelial and smooth muscle cells, retinal microvascular endothelial cells (RMEC), as well as the diseases′ applications of these cells.

Key Words: Endothelial cells, Smooth muscle cells, Atherosclerosis, Diabetic retinopathy

Human umbilical vein endothelial cells (HUVEC)

Human umbilical cords are easily available, whereas human aortic tissue can only be obtained during surgical or invasive interventions. At the time of delivery, when the umbilical cord is cut and clamped, the arteries constrict much more than vein. Thus, it is possible to employ the umbilical vein as a source of endothelial cells (EC).[1)]

The first successful isolation and characterization of EC from the umbilical vein in culture had been reported in 1973 and 1974

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by Jaffe et al[2] and Gimbrone et al[3] respectively. The isolated HUVEC are positive for von-Willibrand factor, a classic endothelial marker, and contain Weibel-palade bodies.[1-3] These cells also form capillary-like structures as a result of angiogenesis when seeded on Matrigel, thus proving their functionality.[1]

The ability to culture EC allows investigators to manipulate -in a controlled manner-the extracellular environment and to study cell biology in far greater detail. Among the seminal findings of that time is the observation that incubation of the cultured EC with inflammatory mediators or bacterial products induces proadhesive, antigen-presenting and procoagulant activities, a phenomenon that is termed "the EC activation".[4-10] The EC derived from the HUVEC have been the major source of the primary EC, mainly because umbilical cords are readily available and ethically unproblematic. The HUVEC have been used to study a range of important patho-physiological processes, including immune-endothelial interactions, endothelial dysfunction related to atheroma formation and tumor metastasis. The literature is filled with studies using the HUVEC model. Since suitable culture conditions have been defined by Jaffe et al,[2] the HUVEC are commonly used to study many important biological processes as follows: (i) Leukocyte-endothelium adhesion, which is a key step towards lymphocyte recirculation, immune cells migration towards inflamed tissues or vasculitis,[11] (ii) Leukocyte transmigration through blood vessels,[12, 13] (iii) Cross-talk between endothelium and immune cells, resulting in the expression of adhesion molecules and cytokines,[14-16] (iv) Atheroma, which is a major pathological phenomenon in developed countries and is largely dependent on both metabolic and inflammatory events,[17] (v) Metastasis formation,[18] which is influenced by adhesive interactions between circulating tumor cells and endothelial cells.[19]

Aortic endothelial and smooth muscle cells

Human aortic tissue can only be obtained from surgical or invasive interventions. Hence, EC and smooth muscle cells (SMC) are mainly separated from the aorta of bovine, porcine, and murine species.[20-22] Particularly, the EC and SMC isolated from mouse are widely used because of wild type mice vs knockout mice models′ availability for developing new therapeutic reagents of a representing vascular disease such as atherosclerosis.[23]

The aortic wall contains 3 layers, consisting of the tunica adventitia, the tunica media, and the tunica intima. The pure isolation of the EC and SMC from these layers is easily possible by the treatment of type I or type II collagenase solutions, and so on. The isolated EC are positive for CD31

endothelial marker and contain their characteristic Weibel-palade bodies. The SMC are positive for α-smooth muscle actin and include actin filaments in their cytoplasm.[23]

Multipotent vascular progenitor cells do exist in the human fetal aorta wall and they are the source of the EC and SMC. The progenitor cells may have implications in the physical and pathological conditions of the vessel. Because these cells can be expanded in culture without obvious senescence for more than 30 population doublings, they may be an important source of the EC for cellular pro- or anti-angiogenic therapies.[24] The progression of diabetes is associated with profound endothelial dysfunction. Diabetes imparts diffuse endothelial perturbation in primary venous and aortic endothelial cells of type I diabetic mice.[25]

Cellular proliferation is involved in the pathogenesis of vascular proliferative diseases such as primary atherosclerosis and restenosis after angioplasty.[26] Whereas early events in atherosclerosis are characterized by an altered endothelial function and by the recruitment of mononuclear leukocytes to the intima, the progression of atheroma involves the proliferation of the SMC, their migration from the underlying media to the intima and their production of extracellular matrix macromolecules.[17, 26] The SMC in atherosclerotic plaques are exclusively derived from the local vessel wall in the apoE knockout mouse model of atherosclerosis.[27] The proliferation of the SMC in vitro atheroma is associated with a decrease of caveolin-1 expression using the wild-type mice vs caveolin-1 knockout mice model.[28]

Retinal microvascular endothelial cells (RMEC)

EC from microvascular regions have many different characteristics compared with the EC from great vessels. Since many general diseases, such as hypertension and diabetes, induce retinopathy, RMEC are ideally used as the subject for microvascular research.

A method for the isolation of the pure homogeneous RMEC population from goat's eye is possible without using multi-step procedure and sophistigated instrument facilities. The RMEC from goat is simply isolated using enzymatic method. They are identified as the EC by morphology and confirmed by positive immunocytochemistry for von-Willibrand factor and CD-31, specific markers for EC.[29]

The RMEC dysfunction is intimately involved with the pathogenesis of many common and severe retinal vascular proliferation disease, particularly in retinopathy of prematurity, ischemic retinal vein occlusion, diabetic retinopathy, and so on. Angiogenesis, the formation of new vessels from pre-existing capillaries, is the common pathologic process of these diseases. Although these

diseases are well characterized clinically, our knowledge of the understanding molecular and cellular pathology is insufficient to allow the development of the improved treatment strategies which are needed urgently.[30] Retinal vascularization is very tightly regulated by coordinated interaction of the EC, pericytes, and astrocytes. Balanced production of positive and negative regulatory factors excreted from these cells is recognized as a complex and critical molecular pathologic basis.[31-36]

Perspectives

Primary culture methods of HUVEC, Aortic EC and SMC, RMEC using enzymes have been established by many researchers. These in vitro models are powerful tools systematically to investigate the pathogenesis of related diseases. The purity of isolated cells is confirmed by specific markers according to the type of cells. The maintenance of cultivated cells is also important in vitro without the contamination by other cells such as fibroblasts. To prevent the contamination, for example, endothelial cell growth factor is used to enforce to grow the EC.

The HUVEC form capillary-like structure on the Matrigel, and can be widely used for studying angiogenesis and cancer cell metastasis. The Aortic EC and SMC are related to the atheroma formation in atherosclerosis. In this disease, the EC lose normal function. And the SMC of tunica media migrate in the plaques, then occupy main bulk of the plaques. The RMEC dysfunction induces diabetic retinopathy. Angiogenesis is a pathologic process of this disease.

Taken together, I hope that complex and critical pathologic basis of the HUVEC, Aortic EC, SMC and RMEC-related diseases are explored by using in vitro models of these cells, and that the improved therapeutic reagents of these diseases are invented.

한글초록

암세포의 전이, 죽상경화증, 당뇨성 망막병증과 같은 병적인 과정에서 혈관내피세포는 핵심적인 역할을 담당한다. 죽상경화증의 죽종 형성에 혈관민무늬근육세포가 직접적으로 관여한다. 배꼽정맥, 혈관내벽, 그리고 망막에 있는 이들 내피세포들은 다양한 효소용액들을 이용하여 얻는다. 순수하게 분리된 이들 세포는 내피세포와 관련된 질병의 시험관 내 연구에 있어 중요한 모델이다. 이러한 관점에서 볼 때 대동맥 벽의 중간막에서 분리한 후 배양한 민무늬근육세포도 죽상경화증의 발병을 설명할 수 있다. 이 종설에서는 사람배꼽정맥내피세포(HUVEC), 대동맥의 내피세포 및 민무늬근육세포, 그리고 망막미세혈관내피세포(RMEC)의 분리 뿐 만 아니라 이들 세포를 이용한 질병연구에 관한 논문들을 소개하고자 한다.

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