Symposium 8

Cultured Vascular Cells and Atherogenesis

Immortalization of Human Endothelial Cells*

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During the development of cultivation technique of venous endothelial cells from more than 600 human umbilical cords in number, a cell line growing to cobblestone monolayer has been estab-

lished and maintained for more than 3 years (166 generations) (Fig. 1). This strain designated ECV304 was characterized by a high proliferative potential in 10%FBS-containing M199 without

HUVEC
×245

ECV304
×245

Fig. 1 Morphology of monolayers.

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Fig. 2

any specific growth factor supplement, an anchorage dependency with contact inhibition, a human chromosomal constitution with a high trisomic karyotype (mode 80), nude mouse tumorigenicity and induction of angiogenesis in rabbit cornea test. Although one of the prominent endothelium markers—Factor VIII related antigen¹⁾—was not detectable, the presence of angiotensin converting enzyme²⁾ and Weibel-Palade bodies³⁾—characteristic of cytoplasmic organelle in endothelium—(Fig. 2), positive immunocytochemistry for the lectin *Ulex europaeus* I⁴⁾, negative staining for alkaline and acid phosphatase as well as several monoclonal anti-human monocyte antibodies, in addition, lack of the mesothelial and epithelial marker keratin⁵⁾, suggest that ECV304 has been originated from umbilical vein endothelial cells by spontaneous transformation but from neither histiocytes nor mesothelial cells. Ultrastructurally, none of the virus-like particles has been detected intracellularly. Human cell lines have been known to exhibit very low frequencies of spontaneous transformation. However, Factor VIII related antigen-poor variant endothelium has been reported to appear not only in the atherosclerotic

but also in the healthy arterial endothelial cells in culture⁶⁾. Monoclonal theory assumes that each atherosclerotic plaque is derived from a single mutationally transformed smooth muscle cell⁷⁾. This work is a report on one case of a phenotypic alteration of normal venous endothelial cells of human origin *in vitro* and the generation of a transformant with indefinite life spans.

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