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ORIGIN OF SPINDLE-SHAPED CELLS IN KAPOSI SARCOMA

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ABSTRACT

Adult skin microvascular endothelial cells derived from new born foreskin were grown and maintained in tissue culture with and without dibutyryl cyclic AMP (DC-AMP) and isobutyl methyl xanthine (IMX). Whereas in the presence of DC-AMP and IMX, the cells showed the typical cobblestone appearance of endothelium, in the absence of these agents the cultured cells permanently converted to a spindle-shaped configuration. Because this phenomenon of transdifferentiation also occurs in the presence of specific cytokines, the profile of which is notoriously altered in acquired immunodeficiency syndrome (AIDS), the findings support the concept that in Kaposi sarcoma the spindle-shaped cells derive from dysfunction in the microvascular environment.

One of the new developments in understanding the biology and molecular biology of the microvascular system is the unusual range of physiological responses in which the microvascular endothelial cell participates. In 1898, Mallory was one of the first pathologists to suggest that tissue phagocytes derive from the endothelium (1). In 1985, Beranek and coworkers obtained evidence that the hyperproliferative mesenchymal cells in fibrosis may also derive from the endothelium (2). Whereas it has been frequently suggested that the spindle-shaped cells in Kaposi sarcoma result from a dysfunction in the microvascular system conclusive evidence to prove this hypothesis has been difficult to obtain. In this report, we describe the biologic potential and the factors that control the morphology and function of skin microvascular endothelial cells, and use this information to provide a possible biological explanation for how the spindle-shaped cells in KS develop from normal microvascular endothelial cells when exposed to an abnormal cytokine and hormonal environment.

Isolation and Growth of Normal Skin Microvascular Endothelial Cells

In 1980, our laboratory reported a method to obtain skin microvascular endothelial cells from the newborn foreskin (3), and in 1983 from adult tissue (4). These procedures have been reviewed (5). Using these methods endothelial cells can now be isolated from the microvasculature of both normal and diseased skin and maintained as functional endothelial cells for up to eight subpassages. In contrast to other cells present in the skin, endothelial cells require a specialized growth environment which both controls their rate of proliferation and their morphology.

Control of Endothelial Cell Morphology

In the presence of cytokines or growth factors that elevate the intracellular levels of cyclic AMP, microvascular endothelial cells maintain a characteristic epithelioid morphology (6,7). This phenotype is identified by the presence of tight junctions and a characteristic and unique actin and vimentin

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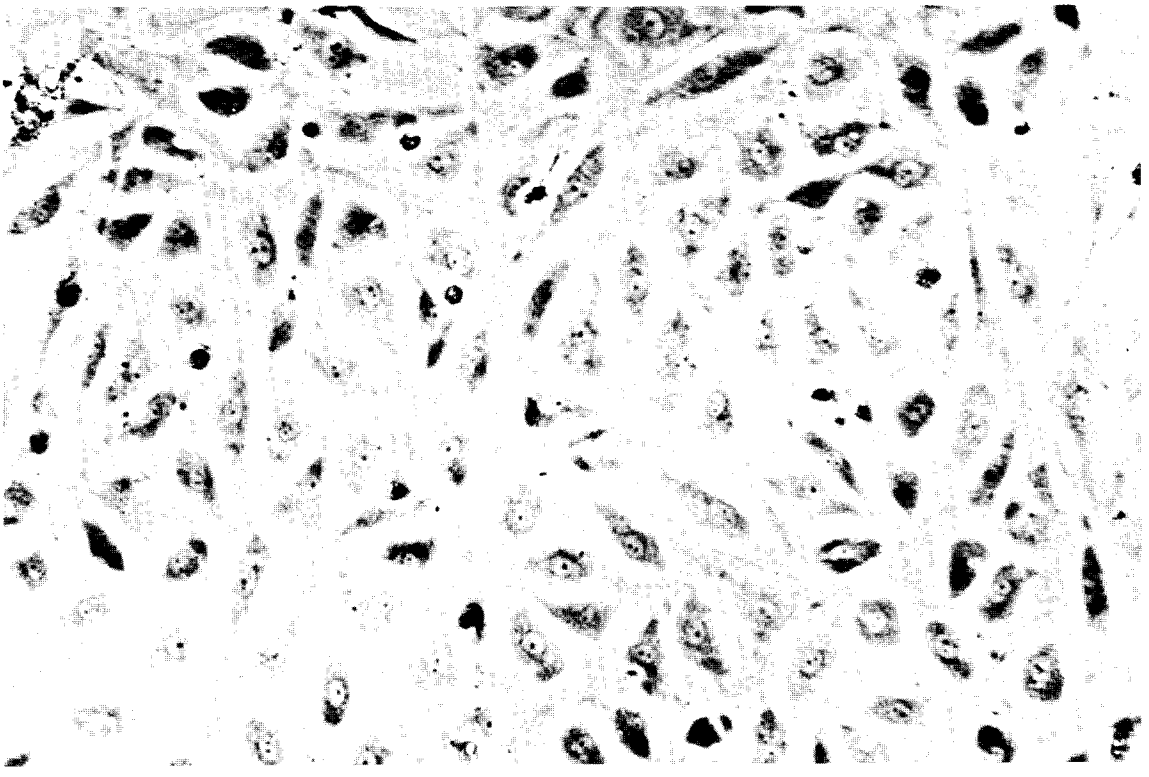

Fig. 1. Phase contrast photomicrograph of an adult skin microvascular endothelial cell population grown and maintained in the presence of dibutyryl cyclic AMP and isobutyl methyl xanthine. Cells show the cobblestone appearance typical of microvascular endothelial cells in the configuration required for homeostasis. (x310).

cytoskeletal network (8,9). Actin is present as dense bundles surrounding the periphery of the cytoplasm, while the vimentin network surrounds the nucleus in a circular array of fine fibrils. Because the actin filaments surround the periphery of the epithelioid cell rather than arranged longitudinally, endothelial cells in this configuration cannot migrate unidirectionally in the way that keratinocytes and fibroblasts migrate in response to injury. In the epithelioid configuration, cells synthesize a classical basement membrane consisting of laminin and type IV collagen and synthesis of type I collagen cannot be detected (10,11).

In the presence of cytokines or hormones which increase intracellular calcium concentration (9), or in the absence of cyclic AMP (6,7), endothelial cells respond by a contraction of the plasma membrane and a reorganization of the cytoskeletal network to allow the cells to assume a spindle-shaped appearance. The endothelial cells thus become primed to participate in the first stages of angiogenesis *in vivo* where the sprouting, migrating cells are spindle-shaped in morphology.

Transdifferentiation of Endothelial Cells

Phase contrast photomicrographs of the two major types of endothelial cells, epithelioid and spindle-shaped, are illustrated in *Figs. 1 and 2.*

If the inflammatory or hormonal stimulus is temporary, transitional epithelioid cells revert to the epithelioid configuration essential to maintain homeostasis when the stimulus is removed. However, when chronically

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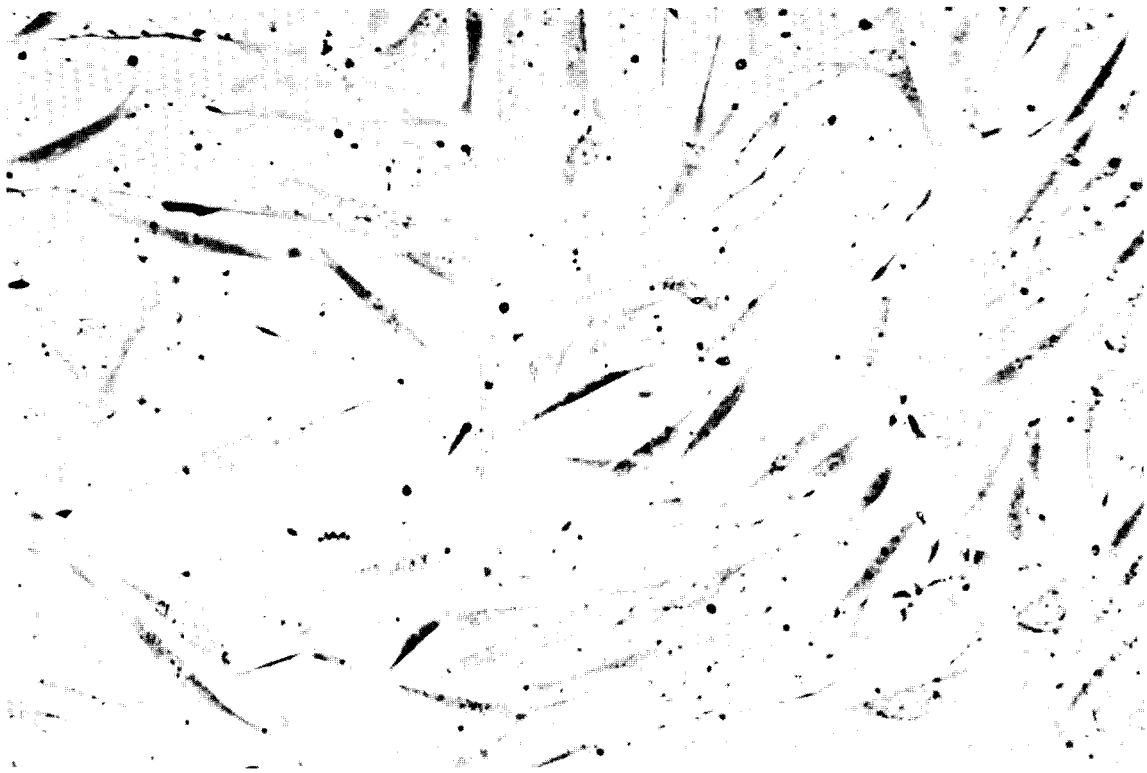

Fig. 2. Phase contrast photomicrograph of an adult skin microvascular endothelial cell population grown and maintained in the absence of dibutyryl cyclic AMP and isobutyl methyl xanthine. The epithelioid cells have completely converted to a permanent spindle-shaped phenotype. (x310).

stimulated, a permanent conversion of an epithelioid cell type to a spindle-shaped cell type takes place. This change in phenotype is an example of transdifferentiation. The term transdifferentiation is defined as an alteration in the phenotype of a cell that has been characterized by an alternative and stable cell morphology. The transition occurs in the absence of cell division.

In KS, the same series of cellular changes observed *in vitro* can be observed in each of the stages of the syndrome *in vivo*. In the granulomatous stage, transitional cells with the characteristics of both endothelial cells and spindle-shaped cells can be detected. In the later stages of growth, spindle-shaped cells, as *in vitro*, lose all phenotypic markers of endothelial cells (e.g., Weibel-Palade bodies) and acquire characteristics of the macrophage cell lineage (e.g., Factor XIIIa and CD-31). On the basis of these changes, it has been suggested that the spindle-shaped cells derive from the bone marrow. However, it is now clear that under the appropriate stimulatory environment, endothelial cells have the capacity to duplicate all of the cellular phenotypes present in the lesion, including the acquisition of Factor XIIIa specificity (12).

What are the factors in HIV infected individuals that cause this dysfunction in the endothelium? Since inflammatory cytokines can produce an increase in endothelial cell transdifferentiation and since human immunodeficiency virus (HIV) infected T cells alter the cytokine profile in serum, we have begun to develop model systems in which both the direct effect of HIV on endothelial cells, the effect of inflammatory cytokines and HIV,

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and HIV and other viruses such as cytomegalovirus (CMV) can be analyzed. Using these *in vitro* models, further analysis of the interaction of inflammatory cytokines, hormones, and other factors present during HIV infections will be possible.

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