ONCOLOGY

Activation of Human Corneal Endothelial Cell Proliferation in Malignant Tumors of Various Locations and in Diabetes Mellitus

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Angiogenic growth factors are peptides of molecular weights from 200 to 10,000 D which were originally isolated from tumor tissues and later from various other tissues including the retina, corpus luteum, and synovial fluid in arthritis [10]. They have been shown to be normally present in an inactive state at cell surfaces in the intercellular matrix of most tissues [3] and to be activated during regeneration processes [4]. Growth factors (GF) activate not only the proliferation of capillary endothelium. Fibroblast GF, for example, is a broad-spectrum mitogen that stimulates the proliferation of tissues of neuroectodermal and mesodermal origin [4]. Activity of these factors is detectable in tissue culture and a vasoproliferative reaction of extracts from certain tissues can be observed on the chorioallantoic membrane of chick embryos and on rabbit cornea.

The endothelium (posterior epithelium) of the human cornea normally displays a very low mitotic activity. The loss of cells with age or as a result of damage is made up for through hypertrophy of cells and their migration to adjacent areas devoid of cellular elements [5]. GF have been shown to activate the proliferation of human corneal endothelium in tissue culture [8,11], but there is very little evidence indicating that they also activate human corneal endothelial cell proliferation *in vivo* [14].

Since the synthesis and activation of GF are characteristic for malignant tumors and other types of pathology (such as proliferative diabetic retinopathy), accompanied by active growth of newly formed vessels [3], we undertook the present study to appraise the level of human corneal endothelial cell proliferation in malignant tumors of various locations (both inside and outside the eye) and in diabetes mellitus.

MATERIALS AND METHODS

Corneal endothelia from 205 human eyes were used. Group 1 comprised 30 eyes enucleated for choroidal melanoma in patients aged 40 to 65 years; group 2, 90 eyes from donors aged 40 to 80 years who had died of breast, lung, stomach, intestinal, uter, or laryngeal cancer; group 3, 60 eyes from donors aged 60 to 81 years who had

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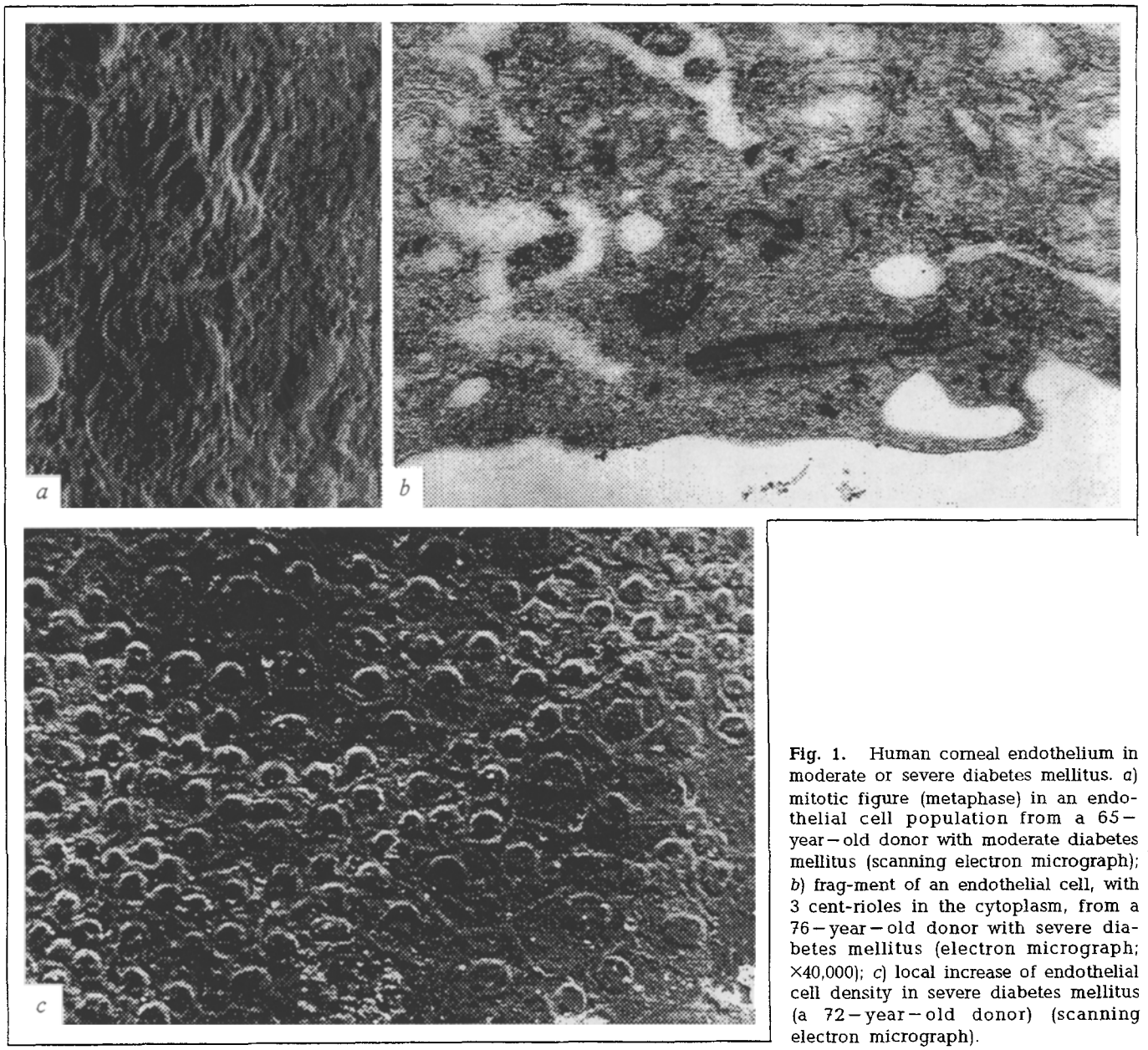

Fig. 1. Human corneal endothelium in moderate or severe diabetes mellitus. *a*) mitotic figure (metaphase) in an endothelial cell population from a 65-year-old donor with moderate diabetes mellitus (scanning electron micrograph); *b*) frag-ment of an endothelial cell, with 3 cent-rioles in the cytoplasm, from a 76-year-old donor with severe diabetes mellitus (electron micrograph; ×40,000); *c*) local increase of endothelial cell density in severe diabetes mellitus (a 72-year-old donor) (scanning electron micrograph).

suffered from diabetes mellitus of various degrees of severity and had died of cardiovascular disease. The 4th, control group, consisted of 25 eyes from 40- to 80-year-old donors who had died of cardiovascular disease or accidental trauma.

The corneas with a strip of sclera 1 mm wide were excised, and flat endothelial preparations were made and impregnated with silver nitrate according to Smolin [13]. However, in order to obtain a suitable flat preparation of human cornea, we had to increase the time of cornea fixation in methanol to 24 h and then to separate the endothelial layer together with Descemet's membrane. In making flat endothelial preparations, which were later stained for DNA by the Feulgen method, whole corneas were prefixed in Carnoy's fluid for 20 min, and the endothelial layer was separated with Descemet's membrane and fixed in Carnoy's fluid for 2 h. Morphometry was performed on a Morphomat-10 instrument (Opton), measuring the density of endothelial cells per mm^2 and cell areas. The number of cells of various sizes was counted per 100 endothelial cells. The results were treated statistically using the Wilcoxon-Mann-Whitney test. The endothelia were examined under scanning and transmission electron microscopes.

RESULTS

Mitotic figures were most frequently found in corneal endothelial cell populations from donors with

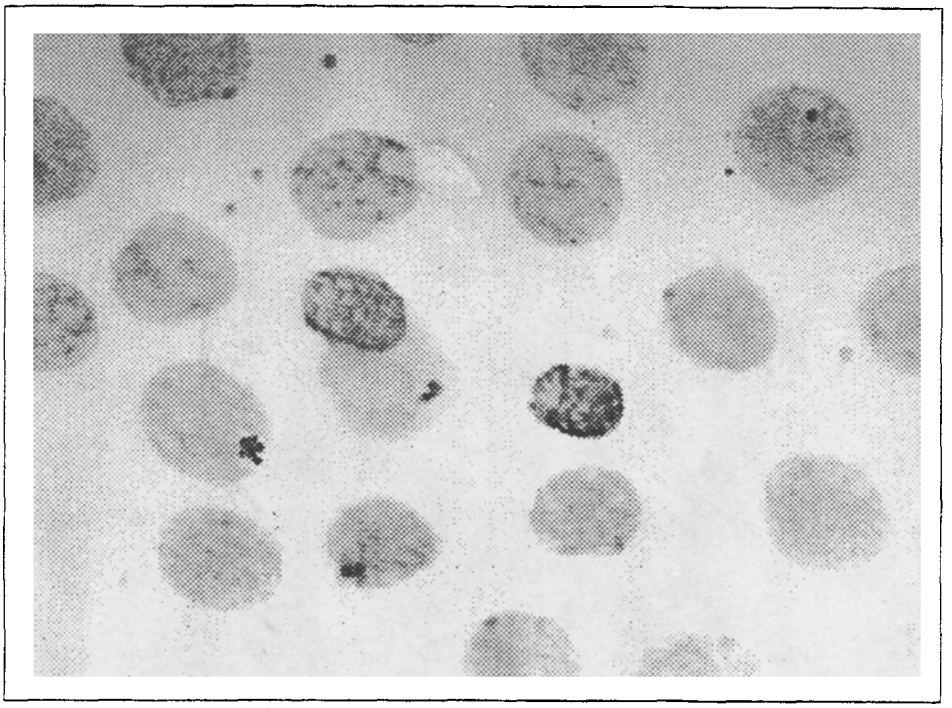

Fig. 2. Mitotic figure (prophase) in a corneal endothelial cell population from a 52-year-old donor with breast cancer. Feulgen's stain for DNA (photomicrograph; ×400).

moderate or severe diabetes mellitus. In their corneas, mitotic figures in various phases of the mitotic cycle were observed, including prophase, metaphase (Fig. 1, *a*), and telophase, as well as anaphase chromosomal bridges. The number of mitotic figures was 2 or 3 per cornea. With transmission electron microscopy, cells with 3-4 centrioles in the cytoplasm were detected (Fig. 1, *b*); the nuclei of these cells were in the G_2 stage.

Mitotic figures - prophase (Figs. 2 and 3), metaphase, and telophase - were also found in endothelial cell populations from donors who had died of cancers of various locations and from individuals whose eye had been enucleated because of choroidal melanoma, but they were encountered much less frequently, especially in donors who had died of cancer - 1 mitotic figure per cornea and only in 5 out of the 90 examined; in melanoma, 1 or 2 mitotic figures per cornea were detected in 10 corneas of the 30 examined.

In the donors with diabetes mellitus, mitotic division was therefore a more active process than

TABLE 1. Endothelial Cell Density and Area, and the Numbers of Large and Small Cells in Endothelial Cell Populations from Various Groups of Donors and from Eyes Enucleated for Choroidal Melanoma

Group	Age, years	Endothelial cell density per mm²	Endothelial cell area, μ²	Number of small cells per 100 endothelial cells	Number of large cells per 100 endothelial cells
Choroidal melanoma	40–65	2416* (2256–2956)	306* (251–318)	14* (11–34.5)	7* (0–15)
Cancers of various locations	40–60	2938* (2600–3829)	264* (239–272)	30.5* (16.0–56.5)	2* (0–2)
	61–80	2272 (1803–2575)	338* (291–370)	29.5* (19–47.5)	13.5* (2.5–23.5)
Moderate or severe diabetes mellitus	60–80	2770* (2680–2870)	290* (268–340)	14* (9–20)	11.5* (9–18)
Control	40–60	2160 (2088–2230)	363 (359–382)	7 (5–10)	23 (19–27)
	61–80	1975 (1926–2094)	399 (350–485)	5 (0.5–10)	25.5 (19–35)

Note. The asterisk indicates a significant difference from the control at $p<0.05$.

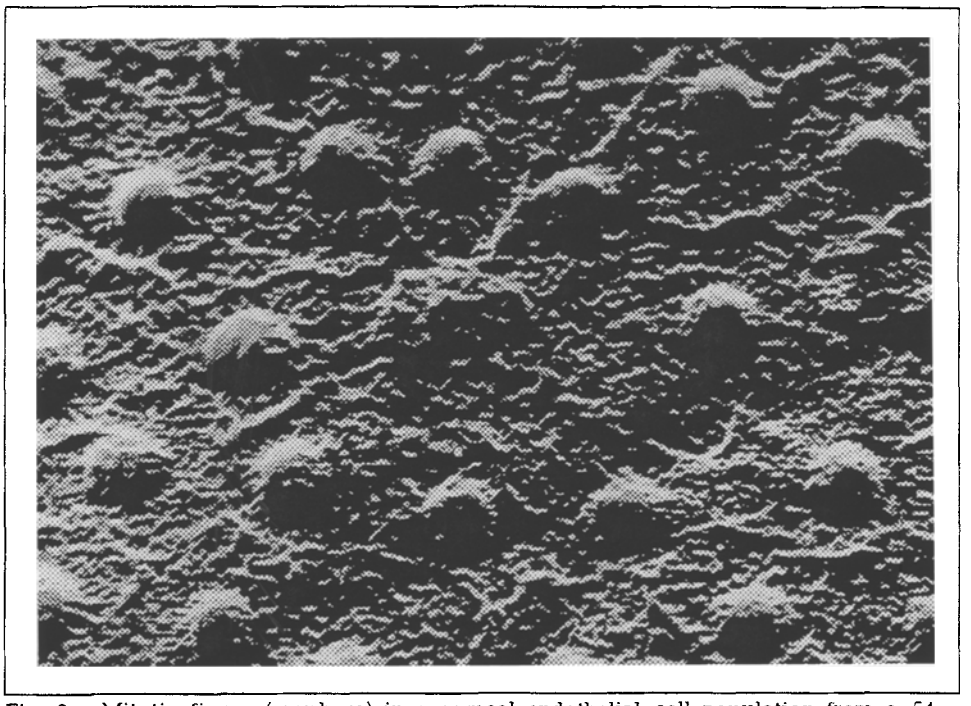

Fig. 3. Mitotic figure (prophase) in a corneal endothelial cell population from a 54-year-old patient with choroidal melanoma (scanning electron micrograph).

in those with melanoma and much more so than in those with cancer. In diabetes mellitus, GF appear to enter the anterior chamber's aqueous humor in greater amounts than in choroidal melanoma. This may be attributed to the damaging action exerted on the retina by products of impaired metabolism in diabetes and to the activated release of vasoproliferative factors from the retina. (Vasoproliferative factors were isolated from retinal extracts [10].) In proliferative diabetic retinopathy, a basic fibroblast GF was detected in the vitreous body [13]. Vasoproliferative factors are integral components of malignant tumors. In patients with such tumors these factors have to overcome the blood-aqueous barrier in order to enter the eye from the bloodstream, whereas in those with choroidal melanoma they are released directly into the eye cavity. Moreover, in cancers of various sites, some effects on the suppression of endothelial cell proliferation may be exerted by immunodepressants and corticosteroids which cancer patients take over prolonged periods.

Epithelial cell density was increased in both moderate and severe diabetes mellitus and in malignant tumors of various locations, including choroidal melanoma (Fig. 1, *c*; Table 1). Age influenced the intensity of proliferative processes in endothelial cell populations in donors aged over 60, although in the older age group only a tendency to increased endothelial cell density was observed and only in cancer patients.

Increased endothelial cell density went along with diminished areas of these cells (Table 1). Increased numbers of small cells (less than 165 μ^2 in area) in endothelial cell populations were consistently present in all three groups of corneal endothelia - those from donors with cancers of various locations and with moderate or severe diabetes and those from patients with choroidal melanoma.

Given that unchanged small cells are products of mitotic cell division, their presence in increased numbers in endothelial cell populations from donors with cancer or diabetes mellitus is an indirect indication of activated endothelial cell division in these disease states.

The number of large cells (550-600 μ^2) was decreased in endothelial cell populations from donors with cancer and those with diabetes mellitus (Table 1).

It follows that intracellular regeneration involving cellular hypertrophy and increases in cell area and in the number of large cells (which is a characteristic feature of human corneal endothelium in health) is reduced when mitotic division of endothelial cells is activated.

That mitotic division in corneal endothelial cell populations is activated in cancers of various locations and in diabetes mellitus is indicated by the present findings that under these conditions endothelial cell density was significantly increased, cell areas were diminished, and the number of small cells rose while that of large cells dropped.

In the available literature, few reports on this subject could be found. Increased (by 11-21%) endothelial cell density was observed under an endothelial microscope in 3 of 11 eyes of patients with choroidal melanoma [15], whereas in eyes of those with diabetes mellitus endothelial cell density was reported to be normal [11].

It has been demonstrated in many studies of animal corneal endothelia *in vitro* and *in vivo* [2,7,9] and of human corneal endothelia in tissue culture [8,12] that the main GF activating mitotic cell division in corneal endothelium are epidermal GF and basic fibroblast factor. An mRNA for epidermal GF and its receptors was identified on cell surfaces as well as an mRNA for fibroblast GF in cell cultures of human corneal endothelium [16]. On the surface of human corneal endothelial cells, receptors for epidermal GF [1] and for basic fibroblast GF [6] were detected. It is these GF which appear to activate mitotic division in the human corneal endothelial cell population.

In human diseases involving the elaboration or activation of GF, these factors apparently enter the eye from the bloodstream (as in cancers of various locations) or occur directly in the eye cavity (as in diabetes mellitus and choroidal melanoma) and induce proliferation of corneal endothelial cells. GF may therefore be regarded as promising agents for activating cell proliferation in damaged corneal epithelium. Also, the very fact that cell proliferation in human corneal endothelium is activated in malignancies and diabetes mellitus may be utilized for diagnostic purposes.

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