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Distribution and Expression of the Adhesion Molecule CD44 on Human Corneal Grafts Is Not Altered by Chemotherapy

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Abstract. *Background/Aim: Acceptance of corneas from donors with a malignancy remains controversial, especially for donors with hematological malignancy. The aim of our study was to examine, for the first time in literature, any structural differences in the integrity of the corneal grafts from donors who have received and from those who have not received chemotherapy. Materials and Methods: The immunohistochemical expression of CD44 was examined in 12 corneal grafts obtained from 8 donors. Three grafts were obtained from 2 donors who had received chemotherapy and the rest were obtained from 6 donors who had not received any kind of chemotherapy. Results: Epithelial cells expressed the CD44 molecule in all grafts of both groups. No CD44 expression was noticed on endothelial cells or in the stroma. Conclusion: Tumorigenesis and the consequent chemotherapy did not affect the structure and integrity of the corneal tissue in the examined samples. We suggest that corneal grafts from cancer donors are safe and functionally equals to grafts obtained from non-cancer donors.*

Corneal opacity is the fourth cause of vision impairment worldwide; with a 3.2% estimation of global blindness could be attributed to this cause (1). Corneal transplantation is the only surgical treatment of restoring vision in patients with corneal opacities. Unfortunately, there is an imbalance between demand and supply of healthy donor corneas (2, 3). Additionally, acceptance of corneas from donors with a malignancy remains controversial, especially for donors with hematological malignancy.

The Eye Bank Associations of America and the European Eye Bank Association accept corneal grafts from donors with a solid tumor, but they recommend avoiding the use of grafts from donors with lymphoproliferative disorders, leukemia, malignant tumors of the anterior and posterior segment (4, 5).

CD44, the hyaluronan receptor (6), is a transmembrane glycoprotein which was recognized as a cell adhesion receptor that plays a key role in cell–cell and cell–matrix interactions, cell migration, lymphocyte homing and activation, tumor growth and metastasis (7-9). The main haematopoietic form of CD44 has a molecular weight of 85-95 kDa (10, 11). CD44 is related with the maintenance of normal tissue structure and the proliferative potency of the epithelium (9, 12, 13).

Hyaluronan, the ligand of CD44, is a glycosaminoglycan component of the extracellular matrix (ECM). Hyaluronan and its metabolites have been implicated in the regulation of angiogenesis (14) and scarless fetal wound repair (15). Moreover, it promotes corneal epithelial regeneration both *in vitro* and *in vivo* (16, 17). CD44 may bind to its ligand in response to antigenic stimuli and may participate in the effector stage of immunological responses (18), and be capable of binding fibronectin, laminin, and collagen I. (11, 19).

Zhu *et al*. showed that, in normal corneas, CD44 was mainly expressed on the membranes of basal epithelial cells, on the keratocytes, and on the vascular endothelial cells of the corneal limbus, but was not expressed on corneal endothelial cells. Additionally, enhanced expression of CD44 was observed on the epithelial cells of corneas with

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Table I. *Demographics of corneal graft donors and haematoxylin-eosin (H-E) staining results.*

Donor	Age (years)	Eye	Chemotherapy	Death-to-preservation time (h)	H-E staining
1	68	Right	No	8:50	Normal structure
	68	Left	No	8:50	Stromal elastosis
2	55	Right	Yes	18:56	Stromal oedema with polypoid projections
3	62	Right	No	13:06	Severe elastosis
	62	Left	No	13:06	Severe elastosis
4	69	Right	No	8:43	Mild stromal oedema
5	74	Right	No	18:23	Normal structure
	74	Left	No	18:23	Normal Structure
6	61	Right	No	19:19	Stromal oedema with polypoid projections
7	65	Left	No	22:40	Severe Elastosis
8	70	Right	Yes	10:03	Stromal oedema
	70	Left	Yes	10:03	Stromal oedema

inflammation and allograft rejection. They reported that, in some corneas with keratitis, dystrophy and keratoconus, the endothelial cells, remained CD44-negative (20).

The aim of our study was to examine, for the first time in literature, whether there are any structural differences in the integrity of the corneal grafts received from donors who had received chemotherapy and from donors who had not received any chemotherapy. For that reason we examined the expression of CD44 in the above categories of corneal grafts.

Materials and Methods

Corneal grafts. Twelve corneal grafts obtained from 8 donors were examined. Three of the grafts were obtained from 2 donors who had received chemotherapy at least one month before corneal donation. The rest of the corneal grafts were obtained from 6 donors who had not received any kind of chemotherapy in their life. All examined corneal grafts were donated from the CorneaGen eye bank (Seattle, WA, USA). Donor demographics are summarized in Table I.

Haematoxylin–Eosin staining. All tissues were fixed in 10% buffered formalin, embedded in paraffin and stained with hematoxylin and eosin (H-E).

Immunohistochemistry. Paraffin-embedded 3-μm thick tissue sections fixed in formalin were prepared. The monoclonal mouse anti-human phagocytic glycoprotein-1 CD44, clone DF 1485 (DAKO, Glostrup, Denmark) was used, diluted 1:50 with DAKO Antibody diluent (DAKO) for 1 h. Each section was subjected to antigen retrieval performed in citrate buffer for 5 min and subsequently, Envision Flex HRP was added for 45 min. Then Envision Flex substrate Buffer plus Envision Flex DAB+ Chromogen was added (1 drop/mlAll incubation steps were performed at room temperature in a humid chamber and followed by thorough washing of the slides with H_2O and Envision wash Buffer (20×) (DAKO). All slides were evaluated by two independent pathologists (X.G & T.C) concerning the tissue structure and the expression of CD44.

Results

Haematoxylin–Eosin (HE) staining. In general terms we did not find any severe structural differences, like a disorganized structure or lack of a cornea layer, amongst the examined grafts (Figures 1 and 2). We only noticed mild structural changes (Figures 3 and 4). Specifically, stromal oedema was observed in 5 corneas, 2 of which had also polypoid projections, elastosis was observed in 4 corneas, and 3 corneas were normal. The results are presented in Table I.

Expression of CD44. Epithelial cells expressed CD44 molecule in all grafts of both groups. Expression pattern was membranous (Figures 5 and 6), while no CD44 expression was found on endothelial cells or in the stroma of both groups.

Discussion

About 30% to 40% of the total number of the corneas available for transplantation come from donors with a malignancy (21-24). Only two cases of cancer transmitted by corneal graft have been described in the literature. The first case was a primary retinoblastoma described by Hata in 1939 (25). The second case was an iris adenocarcinoma described by McGeorge in 1994 (26).

On the contrary, there are large cohort studies that show very low incidence of ocular metastases in corneal donors with active malignancy. Lopez-Navidad *et al*. (24), found that the incidence of malignancy in 204 cancer donors was 1%, 0.6% for solid cancer and 3.7% for malignant hematological disease. Also they did not find any tumor transmission in any of the 325 recipients after a follow-up of an average of 64.1 months (24). Wagoner *et al*. (22), performed a follow-up of 73 patients transplanted with 85 cornea grafts from cancer donors for an average of 10.5 years and they did not record any neoplasia

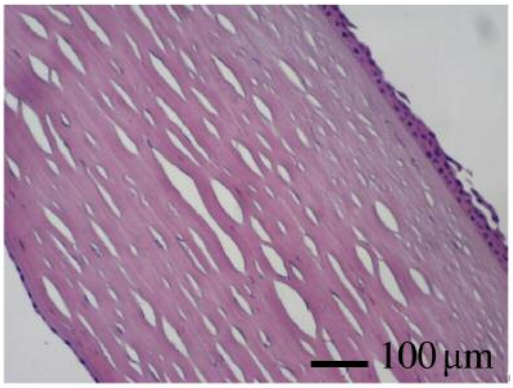

Figure 1. *Haematoxylin-eosin staining in a graft from non-chemotherapy group. The cornea structure is normal. Scale bar=100 µm.*

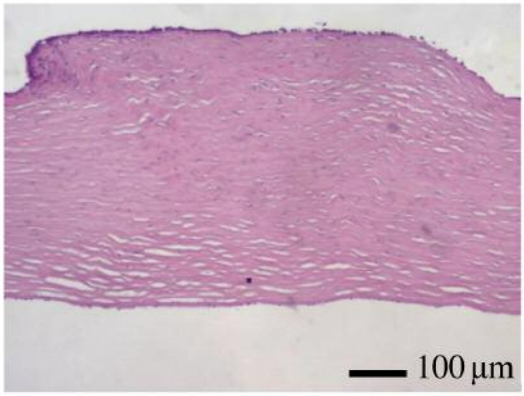

Figure 2. *Haematoxylin-eosin staining in a graft from chemotherapy group. The cornea structure is normal. Scale bar=100 µm.*

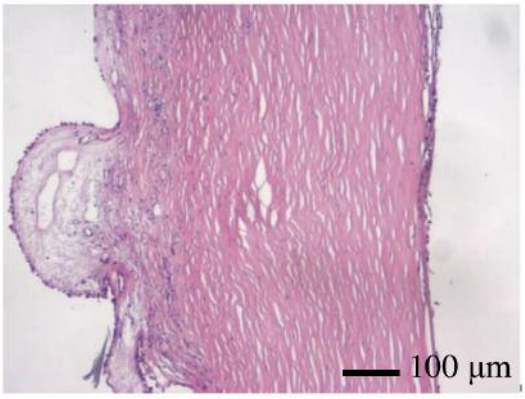

Figure 3. *Haematoxylin-eosin staining in a graft from non-chemotherapy group. Polypoidal projections and stromal oedema are found. Scale bar=100 µm.*

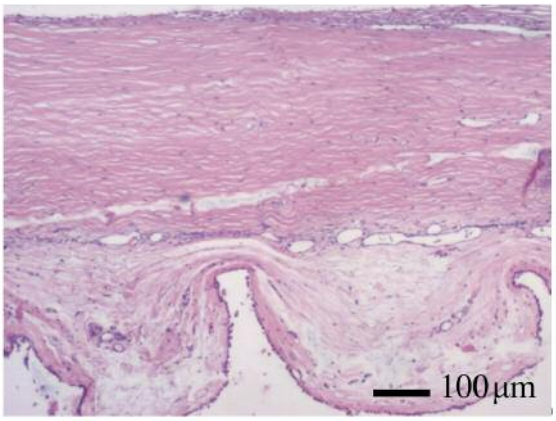

Figure 4. *Haematoxylin-eosin staining in a graft from chemotherapy group Polypoidal projections and stromal oedema are found. Scale bar=100 µm.*

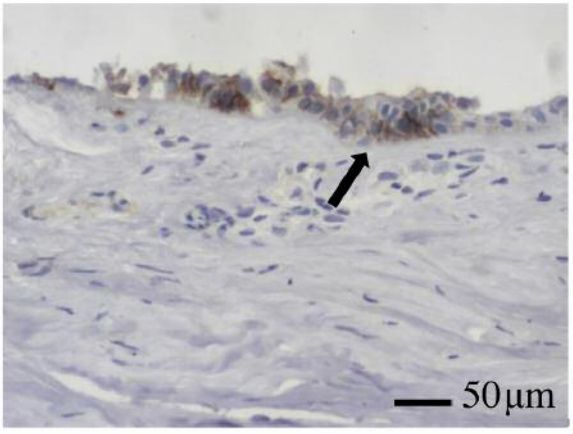

Figure 5. *Membranous expression of CD44 by epithelial cells (arrow) in non-chemotherapy group. Scale bar=50 µm.*

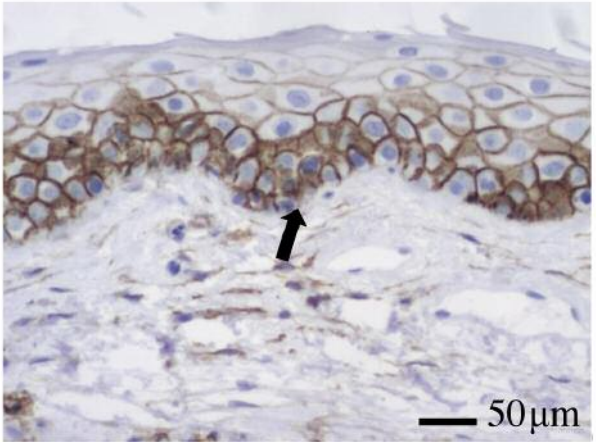

Figure 6. *Membranous expression of CD44 by epithelial cells (arrow) in chemotherapy group. Scale bar=50 µm.*

transmission. Salame *et al*. (23) examined 40 recipients of corneas from cancer donors without finding transmission of malignancy after carrying out an average follow-up of 4 years. Harrison *et al*. (27) transplanted 47 corneas from patients with choroidal melanoma, confined to the posterior pole, and carried out a follow-up of 5.4 years without observing any cancer transmission.

Conversely to the wide range of experimental data regarding the low incidence of ocular metastases in corneal grafts from cancer donors and the very rare incidence tumor transmission in the recipient, less information is available concerning the structural comparison between corneal grafts from cancer and healthy donors.

The present study was the first to examine the structural integrity and compare corneal grafts obtained from donors who had never received chemotherapy and from donors who had received chemotherapy for solid tumors. Specifically, we compared the two corneal graft groups after performing H-E staining and evaluating the immunohistochemical expression of molecule CD44 as described by Zhu *et al*. (20). The H-E staining did not reveal any significant structural differences amongst the examined grafts, except for mild changes which cannot be correlated with any specific factor due to the small number of the examined grafts.

Concerning the expression of CD44, the pattern detected in our study was in accordance with that previously described in normal cornea (20), as well as in various human tissues including skin, intestine, lung and kidney (9, 28). Specifically, we found that epithelial cells were CD44-positive in all grafts of both groups, while, no CD44 expression was detected on endothelial cells or in the stroma. Zhu *et al*. have previously shown that CD44 is expressed on corneal endothelial cells in a number of abnormal conditions including allograft rejection, corneal trauma, primary and secondary endothelial decompensation (20). Therefore, the absence of CD44-positive endothelial cells in both groups supports the notion that the structure and integrity of the examined corneal tissues were not affected by tumorigenesis or by chemotherapy.

In conclusion, the present study was the first to examine the structural integrity of corneal grafts received from cancer and non-cancer donors. The results suggest that tumorigenesis and the consequent chemotherapy received by the donors, does not affect the structure and integrity of the corneal tissue. A limitation of the present study is the small number of cases included. This is due to the fact that the availability of corneal grafts is extremely limited worldwide and for ethical reasons, we did not want to sacrifice any more grafts in an *in vitro* study, especially since we did not find any qualitative or anatomical differences between the grafts examined.

Taking into consideration our results that showed no structural alterations between corneal grafts from cancer and non-cancer donors, as well as previous studies that show extremely rare tumor transmission through corneal grafts from cancer donors, we suggest that corneal grafts from cancer donors are safe and functionally equals with grafts obtained from non-cancer donors.

Conflicts of Interest

None.

Authors' Contributions

Dimtsas G. wrote and revised the manuscript. Grammatoglou X. and Choreftaki T. performed immunohistochemistry, evaluation of slides and revised the manuscript. Dettoraki M. contributed to the revision of the manuscript. Karathanou A. collected data and contributed to the revision of the manuscript. Gouliopoulos N. contributed to the revision of the manuscript. Damaskos C. performed statistical analysis and contributed to the revision of the manuscript. Moschos M. supervised the study and contributed to the manuscript revision.

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