

## MUC4 ASSOCIATION WITH FORMING BLOOD VESSELS DURING CORNEAL WOUND HEALING

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**INTRODUCTION.** MUC4 is a member of the membrane-bound mucin family and the human homologue of the rat sialomucin complex (SMC, rat Muc4) (1). SMC/Muc4 is a well-characterized heterodimeric complex of two subunits ASGP-1 and ASGP-2, derived from a single gene (2). ASGP-1 is tightly but noncovalently bound to the N-glycosylated integral membrane glycoprotein, ASGP-2. The heterodimeric SMC/Muc4 protein has been shown to be expressed in a number of epithelial tissues, including the small and large intestines, trachea, uterus, lactating mammary glands, cornea and conjunctiva (1). In tumor cells, SMC/Muc4 may have multiple functions. ASGP-1 can provide anti-recognition and anti-adhesion properties which protects tumor cells from killing by natural killer cells and promote their metastasis. ASGP-2 acts as an intramembrane ligand for the receptor tyrosine kinase ErbB2/HER2/Neu (3), which regulates phosphorylation and downstream signaling from ErbB2 and plays an important role in development and neoplasia. Recently, our study on human cancer tissues has shown the expression of MUC4 at the endothelial surfaces of blood vessels, which was confirmed in the cultured human endothelial cell lines (4). Studies also showed that the expression of MUC4 is associated with vessel formation during pancreas development in the human fetus, as well as during cell proliferation in human endometrium. The presence of MUC4 at the apical surfaces of endothelial cells raised the questions of whether MUC4 is associated with vessel formation.

**METHODS.** Corneal wounding was produced in rats by cauterization using applicator sticks coated with 75% Silver-Nitrite/25% Potassium-Nitrite. Changes in Muc4 and the endothelial cell protein von Willebrand Factor III (vWF) were assessed by immunohistochemical and immunofluorescence staining of paraffin-embedded sections of corneal tissues after 4, 5, 7, 10 and 15 days post-wounding.

**RESULTS.** Significant thickening of the stratified cornea epithelium was observed at the site of wound 4 days after cauterization. The number of Muc4-expressing cells significantly increased in the corneal stroma, especially around the site of wound and along the region from the limbus to the wound site during the first week after wounding. The Muc4-expressing cells at the wound site appeared in clusters and did not show detectable vWF expression. The endothelial cells, indicated by vWF staining, appeared in the cornea stroma to migrate toward the wound from the limbus individually, in strands or hollow structures. By 10-15 days, loops of these hollow strands form a network of new vessels, which become connected with the blood circulation. The corneal endothelial layer showed thickening in some regions, and vessels were developed. The Iris appeared

nestled up against the corneal endothelium and its vessel density increased. Co-localization of Muc4 expression and the endothelial cell marker, von Willebrand Factor III (vWF), was observed in many of these forming vessels during this process of angiogenesis.

**DISCUSSION.** . To further elucidate the expression and function of MUC4 in normal vasculargenesis and angiogenesis, we assessed the expression pattern and possible function on rat cornea neovascularization during wound healing. This neovascularization is a normal and controlled process. Muc4 is associated with the earliest stages of the recruitment of endothelial cells and formation of blood vessels in response to wound healing in the cornea. These results may have implications for the role that MUC4 may play in endothelial cellular function in normal organ development and tumor progression.

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