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Alterations in Ultrastructure and *c-met* Expression in a Case of Ocular Epithelial Dysplasia Following Topical Mitomycin C Treatment

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Purpose: Topical mitomycin C (MMC) administration is reportedly effective in treating ocular surface neoplasms such as squamous cell carcinoma. We treated a case of ocular epithelial dysplasia that had spread too diffusely to be completely removed. We examined the ultrastructure of and *c-met* (hepatocyte growth factor receptor) expression in dysplastic epithelial cells from this case to evaluate the efficacy of MMC treatment.

Methods: Specimens of dysplastic epithelial tissue from the corneo-limbal region of a 62-year-old man were obtained before and after topical application of MMC. Specimens were examined ultrastructurally and immunohistochemically with an antibody against human *c-met.*

Results: Following topical application of MMC, the dysplastic epithelium exhibited multilayered epithelial cells similar to those seen before treatment. However, ultrastructural examination showed tight interdigitation between neighboring cells, with no intercellular spaces. Also, the marked immunoreactivity to *c-met* in the dysplastic epithelial cells before MMC treatment was decreased after treatment.

Conclusions: Ultrastructural observations indicated a restoration of epithelial cellular differentiation following MMC application. The expression of *c-met* protein was also reduced. Thus, topical MMC was effective in treating epithelial dysplasia of the ocular surface, with no recurrence 15 months post-therapy. **Jpn J Ophthalmol 2000;44:639–642** © 2000 Japanese Ophthalmological Society

Key Words: *c-met*, epithelial dysplasia, mitomycin C, ocular surface, ultrastructure.

Introduction

Epithelial dysplasia can progress to carcinoma in situ or squamous cell carcinoma. While a localized tumor can be easily removed, it is not possible to excise completely a neoplasm of the ocular surface epithelium that involves a larger area of the ocular surface.[1–3] It was recently reported that the topical administration of 0.02% or 0.04% mitomycin C (MMC), an antineoplastic agent, is effective in eliminating neoplasms of the ocular surface that are too diffuse for complete removal.[4,5]

The *c-met* proto-oncogene product is a receptor-type tyrosine kinase.[6] The *c-met* gene was amplified and overexpressed in spontaneous transformance of cultured cells.[7,8] These findings indicate that the overexpression of *c-met* can lead to malignant cellular transformation. The ligand of *c-met* is the hepatocyte growth factor (HGF), a growth factor that is secreted by fibroblastic cells in the regulation of epithelial cell function in many organs. The HGF-*c-met* system is also involved in the maintenance of the integrity of the ocular epithelium.[9]

We previously described details of a case of ocular epithelial dysplasia prior to treatment.[10] We have

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treated this case by topical administration of 0.04% MMC as previously reported because the abnormal epithelium was too diffuse for complete surgical removal. We then reported that, in this patient, the dysplastic epithelial cells showed a reduction in the expression of c-Fos and Fra-2 among the members of AP1 transcription factor after topical MMC treatment,[11] although the exact role of these proteins in the dysplastic epithelial cells have not been clarified. We now examined the ultrastructure of and *c-met* expression in dysplastic epithelium to further evaluate the efficacy of this treatment.

Materials and Methods

Specimens

Details on this patient, a 62-year-old Japanese man, were reported previously.[10,11] Biopsy of the abnormally thickened epithelial tissue with subepithelial neovascularization was obtained from the corneo-limbal region. There was dysplasia of the epithelium of the total ocular surface. The patient was then treated with topical MMC (0.04%) applied 4 times daily for 7 days, as described by Wilson et al.[4] Informed consent was obtained from the patient after explanation of the prognosis of the disease and side effects of this treatment. Ofloxacin was applied 4 times daily to prevent bacterial contamination. Two weeks later, corneal neovascularization and opaque epithelium were observed. The lower corneo-limbal epithelium was biopsied to evaluate the effects of this drug treatment histopathologically. The lesion has not recurred 15 months after treatment.

Ultrastructural Observation

Specimens for TEM transmission electron microscopic examination were fixed in 2.0% glutaraldehyde in 0.1 M phosphate buffer for 24 hours at 4°C, then postfixed in 2.0% osmium tetraoxide for 2 hours at room temperature. Specimens were embedded in Epon 812 (Quetol 812, Nissin EM, Tokyo). Ultrathin sections were stained with uranyl acetate and lead citrate.

Immunohistochemical Study for c-met

Tissue was embedded in OCT compound, and cryosections were cut and fixed with cold acetone for 5 minutes. Sections were allowed to react with a monoclonal antibody against *c-met* protein (Novocastra, UK; ×100 in phosphate-buffered saline [PBS]) after being blocked with 3% skim milk and 5% normal goat serum. After being washed in PBS, the sections were allowed to react with a peroxidase-conjugated secondary antibody. Antibody complexes were visualized with 3,3′-diaminobenzidine as previously described.[9] Negative control staining was performed by omission of the primary antibody. Specimens were counterstained with methyl green and observed under light microscopy.

Results

Ultrastructural Examination

The ultrastructural features of the dysplastic epithelium before the application of MMC were previously reported.[9] In brief, we observed a multilayered proliferation of atypical epithelial cells that exhibited numerous cytoplasmic projections that reached the neighboring cells. Wide intercellular spaces were

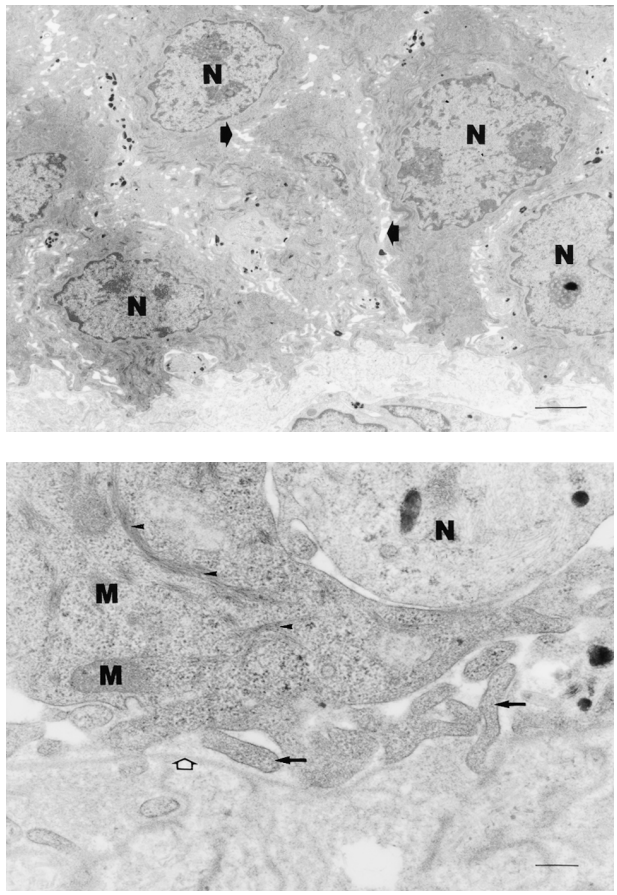

Figure 1. Transmission electron microscopy of cells in dysplastic epithelium before mitomycin C treatment. (**A**) Wide intercellular spaces with prominent cytoplasmic projections (thick arrows) are observed. Bar = 2.0 μm. (**B**) High magnification photomicrograph shows cytoplasmic projections (thin arrows) and basement membrane (open arrow). Cytoplasmic organelles such as mitochondria (M) and intermediate filaments (arrowheads) are also observed. Bar = 0.2 μm. N: nucleus.

present between the neighboring cells (Figure 1). Following the application of MMC 4 times daily for 7 days, comparable specimens continued to exhibit multilayered epithelial cells. However, transmission electron microscopy now showed tight interdigitation between neighboring cells (Figure 2), and a lack of intercellular spaces. In addition, necrotic cells were observed (not shown).

c-met *Expression*

Prior to MMC application, all layers of the dysplastic epithelium were diffusely and strongly positive for *c-met* protein. Following MMC application, the specimens were still multilayered, but were only weakly positive for *c-met* protein (Figure 3).

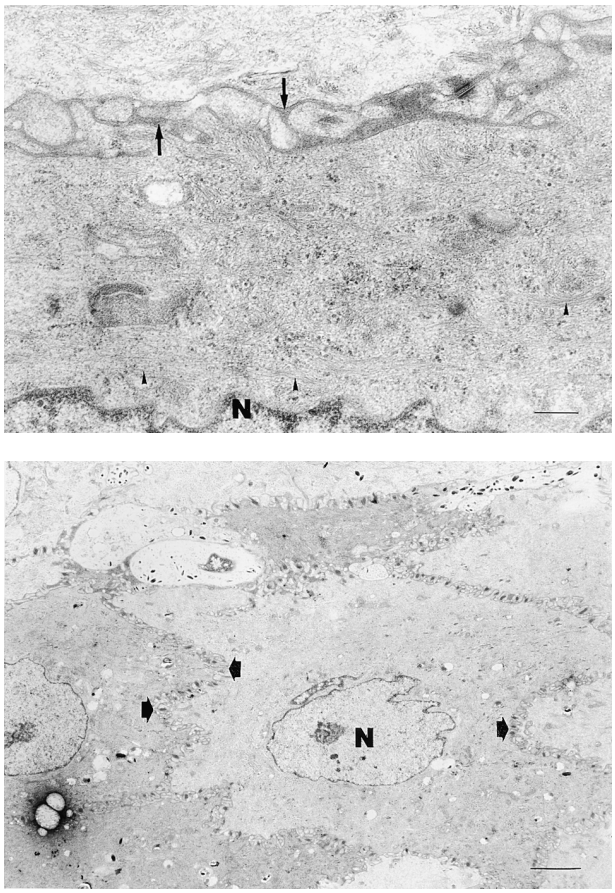

Figure 2. Transmission electron microscopy of cells in dysplastic epithelium following mitomycin C treatment (Figure 1). (**A**) Tight intercellular junctions (thick arrows) are observed. Bar = 2.0 μm. (**B**) High magnification photomicrograph shows tight interdigitation (thin arrows) of neighboring cells. Intermediate filaments (arrowheads) are diffuse and fine in treated cells as compared with untreated cells. Bar = 0.2 μm. N: nucleus.

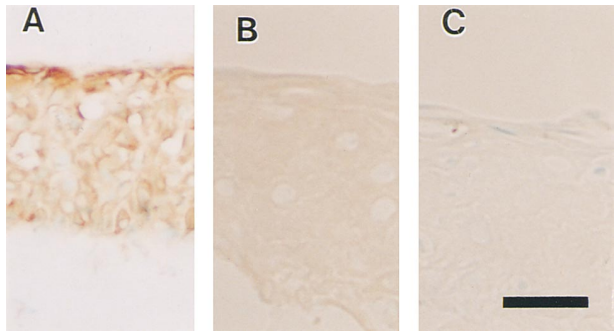

Figure 3. Expression of *c-met* protein (**A**) before and (**B**) 2 weeks after application of mitomycin C treatment. Marked immunoreactivity for *c-met* protein is observed in epithelium before treatment, while only weak immunoreactivity is apparent following treatment. No immunoreactivity was seen for negative control staining (**C**). Bar = 50 μm.

Discussion

Mitomycin C is an antibiotic isolated from a broth, *Streptomyces caespitosis*, and inhibits DNA synthesis in a non-cell cycle-dependent fashion. Topical 0.02% or 0.04% MMC administration is reportedly effective in treating ocular surface neoplasms.[4,5] Sensitivity of dysplastic cells is considered to be heterogeneous; insufficient MMC administration may induce MMC-resistant dysplastic cells. We therefore applied topical administration of MMC at the higher concentration (0.04%) to treat epithelial dysplasia. In the present case, topical MMC demonstrated effectiveness in treating the total ocular epithelial dysplasia, according to ultrastructural findings. Such treatment promoted the differentiation of dysplastic epithelial cells, as confirmed by the development of intercellular junctions. We also observed a decrease in the number of cytoplasmic projections and a narrowing of the intercellular spaces. Decreased activity of cell division following MMC administration might, in part, lead the cells to differentiate.

We also found that the expression of *c-met* was markedly reduced in the dysplastic epithelial cells by topical MMC treatment. HGF, the ligand of *c-met*, is considered to be secreted by subepithelial fibroblastic cells and to be involved in maintaining the integrity of the epithelial tissues. This suggests there was a decrease in the susceptibility to HGF in the dysplastic epithelial cells following topical MMC treatment.

As with other growth factors, the stimulation of the HGF-*c-met* system leads to the expression of AP-1. A decrease in *c-met* expression may have been involved in reducing of the expression of c-Fos and Fra-2 in this case, although the exact mechanism of

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reduction of *c-met* expression is unknown. Findings suggest that ultrastructural examination and study of the expression of *c-met* performed at completion of therapy may be useful in establishing the efficacy of MMC in eliminating the abnormal proliferation of the ocular surface epithelia.

The patient remains free of recurrence 15 months postoperatively. Reported complications from topical administration of MMC include scleral ulceration and perforations.[12] We must carefully follow up this patient to avoid recurrence and also complications.

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