Mitochondrial DNA Mutations in Oncocytic Adnexal Lacrimal Glands of the Conjunctiva

Oncocytic neoplasms are characterized by a marked mitochondrial hyperplasia. They are of epithelial derivation and usually occur in endocrine and exocrine organs such as the thyroid, parathyroids, kidney, pituitary, and salivary glands. Oncocytic lesions of the ophthalmic regions have been reported, especially as oncocytic adenomas of the caruncle. Similar lesions occur rarely in the adnexal lacrimal glands of the conjunctiva. Truncating mitochondrial DNA (mtDNA) mutations in respiratory complex I (CI) subunits have been suggested to be the genetic hallmark of the oncocyic phenotype. The occurrence of such genetic lesions strongly impairs cell respiration and contributes to maintaining the tumor in a low-proliferating, benign state. We describe an oncocyotic adenoma of the lacrimal glands of the conjunctiva harboring mtDNA mutations that cause CI disassembly and associate with a low proliferation index.

Report of a Case. An oncocyotic tumor of the adnexal lacrimal glands of the conjunctiva was collected anonymously. The tumor was partially cystic and composed of cylindrical oncocytic cells with small, ovoid nuclei. It was arranged in a glandular pattern and characterized by an abundant inflammatory stroma with nontumor cells and few lymphocytes, displaying some similarities with Warthin tumor.

Paraffin-embedded sections showed intense cytoplasmic eosin staining of neoplastic cells compared with normal cells (Figure). Staining with a specific antibody against complex V confirmed mitochondria abundance in the neoplastic cells (Figure). Association between the oncocyic phenotype and the presence of truncating mtDNA mutations is generally accepted. To prove that oncocyotic transformation in the ophthalmic region may be unequivocally defined by pathogenic mtDNA mutations, the entire mtDNA sequence was analyzed.

Although the excised tumor mass was very small and mostly composed of tumor cells, microdissection was performed to isolate tumor and normal cells. A clearly pathogenic frameshift mutation (m.14249insC) was detected in the MTND6 subunit of CI (Figure). Because of the physiological polyplody of the mtDNA, mutations may be homoplasmic or heteroplasmic, meaning that all or only some mtDNA copies may be mutated, respectively. The mutation was heteroplasmic in the tumor, although a slight nontumor cell contamination could not be completely excluded. Also, a novel homoplasmic mutation (Human Mitochondrial Database; http://www.hmtdb.uniba.it) was detected (m.12242A>G) in the transfer RNA gene for serine (MT-Ts) (Figure). This mutation was not tumor specific, and positive staining for mitochondria-coded COI (not shown) suggests that the mutation may not affect overall protein translation. Immunohistochemical analysis with an antibody against MTND6 and NDUFB6 subunits of CI showed that MTND6 expression was much fainter in the oncocyic neoplasm compared with nonneoplastic tissue (Figure), in agreement with heteroplasmy of the MTND6 mutation. Although NDUFB6 showed positive staining (not shown), CI assembly is most likely impaired because frameshift mutations in mtDNA-encoded reduced nicotinamide adenine dinucleotide dehydrogenase subunit genes have been shown to result in assembly defects of CI in other oncocytic tumors. Finally, to investigate the association between the occurrence of CI-disassembling mtDNA mutations and a low-proliferating state, Ki67 staining was performed. Indeed, the proliferation index was shown to be 1.8% (Figure).

Comment. To our knowledge, clearly pathogenic mtDNA alterations have never been reported in tumors of the ophthalmic region. The frameshift mutation described here was shown to at least partially hamper CI assembly, likely causing an energetic impairment in oncocyic cells. The occurrence of CI-truncating mutations has been extensively reported in benign oncocyic tumors, where the mutations associate with lack of aggressive behavior. In fact, such mutations render cells unable to stabilize hypoxia-inducible factor 1-α, which is one of the key players in mediating progression to malignancy. The CI structural impairment may hence explain the benign nature of the case described.

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**Figure.** Histochemistry sections and mutations. A, Eosin staining (original magnification ×100). B, Staining of complex V with an antibody anti-ATP5B subunit (original magnification ×100). C, The MTND6 heteroplasmic insertion of C in position 14249 (arrow) of the mitochondrial genome in tumor and in the same patient's nonneoplastic tissue. D, The homoplasmic MT-TS m.12242A>G mutation (arrow) in tumor and in control nonneoplastic tissue (not of the same patient). E, Fainter staining of MTND6 in oncocytic cells (red circle) compared with normal conjunctival cells (black circle) (original magnification ×100). F, Staining with Ki67 (original magnification ×100).

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Possible In Vitro Model of Toxic Epidermal Necrolysis

Toxic epidermal necrolysis (TEN) is an acute, life-threatening, and potentially blinding mucocutaneous disease. It was first described by Lyell in 1956.1 While several factors including a spectrum of medications2 that may play a role in the pathogenesis of TEN have been reported, the disease mechanism remains unknown. The lack of in vitro and animal models is a major obstacle in investigating the pathogenesis and treatment of TEN. The purpose of this study was to develop an in vitro model for TEN.

Methods. A pool of sera from 3 patients with biopsy-proven, acute TEN was used as a reagent in an organ culture. A pool of sera from 3 age- and race-matched healthy individuals was used as a control reagent. Normal human skin (NHS), normal human conjunctiva (NHC), and normal human buccal mucosa (NHBM) were used as substrates in the organ culture. Informed consent was obtained from all patients and healthy individuals. All experiments were approved by an institutional review board.

The in vitro organ culture investigating the effect of TEN serum on normal human tissue was similar to an earlier technique that investigated the effect of sera obtained from patients with pemphigus vulgaris3 or ocular cicatricial pemphigoid.4

After the incubation had been terminated, pieces of NHS, NHC, and NHBM were embedded in paraffin blocks for routine histopathological analysis.

Results. On light microscopy, specimens of NHS, NHC, and NHBM incubated with TEN serum showed morphologic changes consistent with TEN (Figure 1). These changes were observed in all epithelial cell layers of NHS and NHBM after 72 hours and in NHC after 48 hours of incubation. Extensive shedding of the epithelial cells, leaving only the basal layer, was observed in NHS and NHBM incubated in normal human serum for the same period. In addition, a separation between the basal epithelial cell layer and basement membrane zone was observed in NHS and NHBM after 72 hours of incubation. The separation was not observed in NHC during the incubation period of up to 96 hours. On the other hand, unlike NHS and NHBM, areas of frank acantholysis and epithelial shedding were observed in NHC after 48 hours of incubation with TEN serum. The epithelium of NHS, NHC, and NHBM cultured in normal human serum remained intact (Figure 1). Histopathological features in biopsy specimens of skin and conjunctiva from a patient during an acute phase of TEN were similar to those observed in NHS and NHC incubated in the pool of TEN sera (Figure 2).

Comment. The results of this study demonstrate the in vitro effect of sera obtained from patients in the acute phase of TEN on normal human tissues typically affected by the disease. The histopathological changes induced in vitro were similar to those observed in the biopsied tissues of patients with TEN. Necrosis involving the full thickness of the epithelium was observed in NHS, NHC, and NHBM incubated with TEN serum. Similar findings have been previously described in the skin5 and conjunctival6 biopsy specimens of patients with TEN.

Significant pathological findings were observed in NHC after shorter incubation times compared with NHS and NHBM, suggesting that conjunctival epithelium is more susceptible to injury by the pathogenic components in TEN serum. It is possible that this plays a role in induction of chronic cicatizing conjunctivitis, seen in approximately one-third of patients who recover from the acute phase of TEN.8 Unlike conjunctiva, skin and buccal mucosa typically heal within a few weeks after the acute phase of TEN and do not exhibit clinically evident chronic inflammation.