WT1 and Bcl2 Expression in Melanocytic Lesions of the Conjunctiva

An Immunohistochemical Study of 123 Cases

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Objective: Recent studies indicate that WT1 and Bcl2 protein are detected in melanocytic lesions of the skin. We examined, for the first time, WT1 and Bcl2 expression in a variety of conjunctival melanocytic lesions to evaluate their diagnostic utility compared with other melanocytic markers.

Methods: Protein expression and localization of WT1 and Bcl2 were studied by means of immunolabeling and semiquantification in 123 conjunctival melanocytic lesions (71 benign nevi, 21 atypical nevi, 11 primary acquired melanosis, and 20 malignant melanomas). Ancillary immunohistochemical studies were performed with Bcl2, S100, HMB45, and Melan A antibodies.

Results: WT1 showed a graded increase in expression in lesions with increasing atypia. Higher mean numbers of WT1-positive cells correlated with increasing atypia in melanocytes. In all cases, Bcl2 expression was positive and more robust than was S100, HMB45, or Melan A expression. WT1 and HMB45 frequently showed diffuse and strong staining in atypical nevi, primary acquired melanosis with atypia, and malignant melanomas compared with benign lesions.

Conclusions: Bcl2 is a highly sensitive immunohistochemical marker for melanocytic tumors of the conjunctiva; HMB45 and WT1 staining distinguishes benign from malignant lesions.

Clinical Relevance: Our results show that HMB45 and WT1 immunolabeling is helpful in the evaluation of conjunctival melanocytic lesions. Accordingly, we recommend the development of an immunohistochemical panel to classify these lesions.

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To our knowledge, there are no published WT1 and Bcl2 studies in conjunctival melanocytic lesions of the conjunctiva. The objectives of this study are to evaluate WT1 and Bcl2 expression in conjunctival melanocytic lesions and to explore their diagnostic usefulness compared with that of other melanocytic markers.

METHODS

CASE SELECTION

One hundred twenty-three cases of conjunctival melanocytic lesions, including 71 benign nevi, 21 atypical nevi, 11 cases of primary acquired melanosis (PAM), and 20 malignant melanomas, were retrieved from the archives of the Armed Forces Institute of Pathology. All of the cases were biopsy specimens with available paraffin blocks that were diagnosed during the past 10 years and subsequently reviewed histologically to confirm the diagnosis. This study was approved by the Armed Forces Institute of Pathology institutional review board.

IMMUNOHISTOCHEMICAL ANALYSIS

Immunohistochemical analysis was performed on 5-μm, formalin-fixed, paraffin-embedded sections with monoclonal antibodies for WT1 (6F-H2; Cell Marque Corp, Rocklin, California), 1:10 dilution; Bcl2 (Dako North America Inc, Carpinteria, California), 1:50 dilution; HMB45 (Dako North America Inc), 1:50 dilution; Melan A (Ventana Medical Systems Inc, Tucson, Arizona), prediluted; and polyclonal antibody S100 (Dako, Carpinteria, California), prediluted. Tonsil specimens were used as the positive control for Bcl2, skin with known malignant melanoma was used for HMB45 and Melan A, and healthy skin was used for S100.

Antibody reactions were graded as weak (+), moderate (++) and strong (+++), and the overall staining pattern was recorded as focal or diffuse. The expression of Bcl2 protein was identified as cytoplasmic. In cases that were heavily pigmented, we used the red chromogen as counterstain for Bcl2 protein identification. Immunohistochemical analysis for WT1 was performed on selected cases: 10 benign nevi, 6 atypical nevi, 7 PAM with or without atypia, and 7 melanomas. All 123 cases were stained for Bcl2, HMB45, S100, and Melan A.

RESULTS

The immunohistochemical analysis results are summarized in Tables 1, 2, 3, and 4 and are illustrated in Figure 1. The 123 patients in the study ranged in age from 2 to 84 years (mean age, 37.2 years) and included 71 males and 51 females (the designation of the sex of 1 patient was not available).

HISTOPATHOLOGIC ANALYSIS

Of the benign and atypical nevi, 43% were amelanotic and 32% were cystic. Ninety percent of the cystic nevi were benign, whereas only 10% of the atypical nevi were cystic. Balloon cell nevi represented 3% of the cases. Chronic inflammation was present in 33% of nevi, with 29% containing abundant eosinophils, mostly in children (median age, 12 years; range, 6-22 years). Malignant melanomas were frequently accompanied by dense inflammatory infiltrates, especially at the deep margins.

IMMUNOHISTOCHEMICAL ANALYSIS

Representative immunohistochemical images of WT1, Bcl2, and HMB45 expression are illustrated in Figure 1, and graphical data that pertain to WT1 and HMB45 are shown in Figure 2 and Figure 3, respectively. WT1 showed diffuse positive staining in 86% (6 of 7) of the melanomas (Figure 1A) and in 71% (5 of 7) of the cases of PAM, especially those with atypia. Immunolabeling for WT1 was observed in 33% (2 of 6) of the atypical nevi and was locally positive for 80% (8 of 10) of the benign nevi, 67% (4 of 6) of the atypical nevi, 14% (1 of 7) of the cases of PAM, and 14% (1 of 7) of the melanomas. Alternatively, WT1 expression decreased with maturation of the melanocytic elements in benign nevi (Figure 1B).
Diffuse positive staining for Bcl2 was seen in 94% (116 of 123) of all the cases, with focal positivity in 2% of benign nevi (n = 3), 1% of atypical nevi (n = 1), and 2% of melanomas (n = 3). More specifically, Bcl2 showed positive staining in all benign nevi, with a diffuse staining pattern in 68 of 71 cases (96%) and focal expression in 3 (4%) (Figure 1C). For malignant melanoma, there was diffuse staining in 17 of 20 cases (85%) and focal staining in 3 (15%) (Figure 1D).

HMB45 staining was positive in 64 of 71 benign nevi (90%), with diffuse staining in 7 of 71 cases (10%) and focal staining in 57 (80%) (Figure 1E). In atypical nevi, HMB45 staining was positive in 18 of 21 cases (86%), with a diffuse staining pattern in 5 of 21 cases (24%) and focal staining in 13 of 21 (62%). HMB45 staining was focal in all cases of PAM without atypia and was weak in 2 of 3 cases (67%) and moderate in 1 (33%). In PAM with atypia, staining was strong in 7 of 8 cases (88%) and moderate in the remaining case (13%). HMB45 staining was positive in 19 of 20 malignant melanomas (95%), with diffuse staining in 14 of 20 cases (70%) and focal staining in 5 (25%); HMB45 staining was negative in a single case (5%) (Figure 1F). Of interest, S100 protein and Melan A did not differentiate benign from malignant lesions, except for 2 of 20 melanoma cases (10%) that were negative for Melan A.

Melanocytic tumors of the conjunctiva are not rare. In a large series of 1643 conjunctival tumors, 53% were melanocytic and included nevi and malignant melanomas. Despite their frequency, conjunctival melanocytic proliferations continue to pose diagnostic dilemmas. For example, some melanocytic tumors completely lack pigment, which challenges the physician and the pathologist. In the present study, 39% of melanocytic tumors were nonpigmented. To illustrate this potential diagnostic pitfall, one example of a melanotic nevus in the present series was diagnosed as a lymphoid lesion by the physician. Histopathologically, conjunctival nevi may display a unique pattern where inclusions of the epithelium form solid epithelial islands interrupted by cysts. These lesions are regarded as hamartomatous malformations known as cystic nevi. In this study, 24% of nevi were cystic. Although they are readily diagnosed clinically by the use of slitlamp examination, they occasionally cause diagnostic problems for general pathologists. Equally difficult is the separation of tumors of melanocytic lineage that display morphologic variants. Without appropriate immunohistochemical stains, the balloon cell nevus is sometimes mistaken for xanthelasma.

### Table 3. Degree of Positivity for Bcl2, HMB45, S100, and Melan A in Conjunctival Melanocytic Lesions

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<th>Antibody</th>
<th>Grade</th>
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<th>Atypical Nevi (n=21)</th>
<th>PAM Without Atypia (n=3)</th>
<th>PAM With Atypia (n=8)</th>
<th>MM (n=20)</th>
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Abbreviations: MM, malignant melanoma; PAM, primary acquired melanosis; +, weak; ++, moderate; ++++, strong; –, negative.

### Table 4. Degree of Positivity for WT1 in Conjunctival Melanocytic Lesions

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Grade</th>
<th>Benign Nevi (n=10)</th>
<th>Atypical Nevi (n=6)</th>
<th>PAM Without Atypia (n=7)</th>
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Abbreviations: MM, malignant melanoma; PAM, primary acquired melanosis; +, weak; ++, moderate; ++++, strong; –, negative.

**COMMENT**

Melanocytic tumors of the conjunctiva are not rare. In a large series of 1643 conjunctival tumors, 53% were melanocytic and included nevi and malignant melanomas. Despite their frequency, conjunctival melanocytic proliferations continue to pose diagnostic dilemmas. For example, some melanocytic tumors completely lack pigment, which challenges the physician and the pathologist. In the present study, 39% of melanocytic tumors were nonpigmented. To illustrate this potential diagnostic pitfall, one example of a melanotic nevus in the present series was diagnosed as a lymphoid lesion by the physician. Histopathologically, conjunctival nevi may display a unique pattern where inclusions of the epithelium form solid epithelial islands interrupted by cysts. These lesions are regarded as hamartomatous malformations known as cystic nevi. In this study, 24% of nevi were cystic. Although they are readily diagnosed clinically by the use of slitlamp examination, they occasionally cause diagnostic problems for general pathologists. Equally difficult is the separation of tumors of melanocytic lineage that display morphologic variants. Without appropriate immunohistochemical stains, the balloon cell nevus is sometimes mistaken for xanthelasmas.
Another confounding factor for pathologists and physicians alike is the different nomenclature applied to melanocytic lesions of the eye. Ophthalmic pathologists apply the term PAM to refer to intraepithelial proliferation of dendritic melanocytes of the conjunctiva that primarily affects adults. In this context, PAM is divided into low-risk PAM without atypia and PAM with atypia, which reflects the spectrum of biological behavior. Without atypia, PAM does not progress to malignant melanoma, whereas PAM with atypia (malignant melanosis and melanoma in situ) progresses to malignant melanoma in approximately 46% of cases. In a recent study by Shields et al., PAM with atypia progressed to melanoma in 3% of patients, whereas melanoma arose in 13% of patients with PAM with severe atypia.

Figure 1. Representative immunohistochemical images of WT1, Bcl2, and HMB45 expression. A, Diffuse WT1 staining in a melanoma. B, In a benign nevus, WT1 expression decreases with maturation of the melanocytic elements. C, An amelanotic nevus with diffuse Bcl2 staining. D, Diffuse Bcl2 immunolabeling in a malignant melanoma. E, Focal staining for HMB45 in a nevus. F, Diffuse HMB45 staining in a melanoma.
Atypical nevi of the conjunctiva are commonly seen in children and adolescents and are considered borderline melanocytic tumors that show cellular atypia. The diagnostic challenges posed by this entity were emphasized in a previous article. Although immunohistochemical analysis is an important tool in the differential diagnosis of melanocytic lesions, pitfalls are not uncommon. This is particularly true when biopsy samples are small and clinicopathologic correlation is lacking.

In this study, inflammatory infiltrates, mostly lymphocytes, were frequently seen at the deep margins of benign and malignant melanocytic tumors. In these lesions, Bcl2 staining of the lymphocytes did not interfere with detection in the melanocytic elements (Figure 1D). The smaller nuclei of the lymphocytes were readily distinguished from either nevus or melanoma cells. In addition, Bcl2 staining of the lymphocytes did not interfere with detection in the melanocytic elements (Figure 1D).

Significantly, we demonstrated that WT1 immunolabeling was weak to moderate in benign nevi and strong and diffuse in malignant melanomas. This finding remains controversial, but similar results have been described previously. In one study, only 1 of 11 nevi showed faint staining with HMB45, whereas malignant melanomas were mostly positive. Sharara et al also found that HMB45 was expressed in 72.7% of primary melanomas and in 85.0% of melanomas in the context of PAM. Regarding PAM, this group observed that 42.8% of PAM with atypia expressed HMB45, whereas HMB45 was expressed in 11.1% of PAM without atypia and in 8.5% of nevi, consistent with the present findings. To date, there are no published data, to our knowledge, that assess the value of WT1 in melanocytic lesions of the conjunctiva.

The biological composition of melanocytic lesions is not well understood; however, different patterns of gene expression are emerging that offer clues to malignant progression. In this study, Bcl2 expression was positive in all benign and malignant melanocytic lesions of the conjunctiva. Bcl2 expression was more consistently positive than were the other routine melanocytic markers. Not only does this finding provide clues to our understanding of the biological features of this disease, it enhances diagnostic sensitivity in the diagnosis of melanocytic lesions of the conjunctiva.

The results of this study confirm the diagnostic usefulness of combined WT1 and HMB45 in the stratification of melanocytic lesions of the conjunctiva into benign and malignant categories. Bcl2 shows high sensitivity for the detection of melanocytic lesions, and HMB45 demonstrates greater specificity for the identification of malignant melanomas.

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