Characterization of the lymphoid stroma in Warthin’s tumor of salivary gland by immunohistochemistry, heavy chain gene and Bcl-2 gene rearrangement

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Abstract: Warthin’s tumor is rarely associated with malignant lymphoma. Only 18 cases were reported in the literature so far. In most cases the latter is a low grade process, including Marginal zone/Mucosa associated lymphoid tissue (MALT) type lymphoma, follicular lymphoma, and rarely diffuse large cell lymphoma which may arise de novo or secondary to low grade lymphoma. This study was conducted to determine the prevalence of occult B cell monoclones and genetic alterations in Warthin’s tumor. Fourteen cases of Warthin’s tumor were stained with antibodies to CD3, CD20, kappa and lambda light chains. On six cases of randomly selected Warthin’s tumor, polymerase chain reaction (PCR) of IgH gene rearrangement (IgH-GR) was performed on genomic DNA extracted from formalin-fixed paraffin embedded tissue. One case of primary salivary gland indolent B-cell lymphoma and 3 cases of sialadenitis were analyzed by the same methods for comparison. In all Warthin’s tumor and sialadenitis cases most of lymphoid stroma was B cell phenotype and concentrated in germinal centers. T cells were mostly located between germinal centers. No light chain restriction was demonstrable by kappa and lambda immunostains. Molecular genetic studies failed to show IgH-GR by FISH and showed polyclonal by IgH PCR. In contrast, the lymphoma case showed a diffuse proliferation of small B cells with light chain restriction and a minor component of reactive T cells. FISH showed IgH-GR and bcl-2 gene translocation with monoclonality by IgH PCR. Our study concludes that the lymphoid stroma of Warthin’s tumor is reactive.

Key Words: Warthin’s tumor, lymphoid stroma, immunohistochemistry, gene rearrangement

Introduction

Warthin’s tumor is the second most common benign neoplasm of parotid glands and accounts for 5% to 11% of primary parotid neoplasms. It reveals a varying component of bilayered oncocytic columnar and basaloid epithelium with a lymphoid stroma. The epithelium commonly lines cystic spaces and forms numerous papillations. The stroma may be abundant and often contains lymphoid follicles with reactive germinal center. Malignant transformation in either epithelial or lymphoid component in a Warthin’s tumor is extremely rare. And malignant transformation in the latter is rarer than the former [1]. It has been reported that Warthin’s tumor is rarely associated with malignant lymphoma. Most of these were low-grade lymphomas, including MALT-type lymphoma and follicular lymphoma. They are also associated with diffuse large B cell lymphoma, Hodgkin’s disease, and T-cell lymphoma [2-17]. This relationship between Warthin’s tumor and lymphomas gave the impression that these lymphomas might arise in the lymphoid stroma of Warthin’s tumor. The immunophenotypic and genetic characters of the lymphoid stroma in Warthin’s tumor was rarely described in the literature and has remained uncertain. As an initial step to resolve this question, we
investigated cases of Warthin’s tumor in search of occult B-cell monoclonal and genetic alterations.

**Materials and methods**

The surgical pathology laboratory information system was searched for cases of Warthin’s tumor of salivary gland origin over the past five years. A total of 14 such cases were identified. In each case, all available pathologic materials related to the salivary gland biopsy were reviewed, and the diagnosis was confirmed. One case of primary salivary gland lymphoma (follicular and diffuse, predominantly small cleaved cell type-grade I) and 3 cases of sialadenitis were also selected for comparison.

Representative formalin-fixed paraffin-embedded tissue sections were selected, stained with antibodies to CD3, CD20, kappa and lambda light chains using a conventional Immunohistochemical autostainer (Dako, Inc.) and were analyzed by the quantitative Automatic Cellular Imaging System (ACIS, Chromovision). The percentage and intensity of the staining cells were reported.

Presence of immunoglobulin heavy chain (IgH) rearrangement and bcl-2 gene translocation was evaluated by molecular cytogenetics.

Two color fluorescence in situ hybridization (FISH) was employed for analysis. DNA probes were prepared according to the manufacturer’s protocol. The probe designated for this translocation is a locus specific (Vysis, Inc., Downer’s Grove, IL) IGH Dual Color, Break Apart Translocation Probe which is a mixture of two probes that hybridize to the opposite sides of the joining (J) through constant (C) regions of the IgH locus. All paraffin embedded tissue sections were deparaffinized and pretreated prior to hybridization with the probe. Immediately prior to hybridization, the slides were dehydrated in an ethanol series of 70%, 85% and 100% for one minute each and air dried. The FISH probe mix (10ul) was added to the tissue section and coverslipped. Target DNA and the FISH probe were co-denatured at 37°C for 6 minutes using a commercial hybridization system (Hybrite, Vysis, Inc.) and subsequently incubated at 37°C overnight. Slides were washed with 0.4xSSC/1%NP40 for 2 minutes at 73°C. The cells were counterstained with diamidino and viewed using a fluorescent microscope (Leica DMRB) outfitted with appropriate filters for multicolor FISH analyses (Quips PathVysion, Applied Imaging Corp., Inc.). A minimum of 200 cells were evaluated and scored for each probe per slide.

Polymerase chain reaction for IgH gene rearrangement (IgH PCR) was performed on all six cases of randomly selected Warthin’s tumor using genomic DNA extracted from formalin-fixed paraffin embedded tissue. Reactions were evaluated using an automated thermocycler (Perkin Elmer 2400). We analyzed 10ul of the final PCR product using 1.5% agarose gel electrophoresis and visualized the product with ethidium bromide staining as one reproducible dominant band in the expected size range of 80 to 120 base pairs. Positive and negative controls consisted of extracted DNA from known primary salivary gland lymphoma case and water, respectively.

**Results**

Parotid gland specimens with Warthin’s tumor were obtained from 14 patients (10 women, 4 men). Patient’s ages ranged from 48 to 89 years with a mean of 58 years. Twelve patients had total or partial parotidectomy for a parotid gland mass. Two patients including an autopsy case were diagnosed from neck masses. A 58 year old female who had Sjogren’s syndrome and developed indolent B-cell lymphoma as well as three cases of chronic sialadenitis (2 females, 1 male; mean age 58 years) were analyzed for comparison. The lymphocytic component of Warthin’s tumor cases varied. Reactive germinal centers were present in most cases. IHC showed that most of the lymphoid stroma was B cell phenotype (CD20+) and was concentrated in germinal centers. T-lymphocytes (CD3+) mostly were in areas away from germinal centers. Kappa and lambda light chains immnostaining showed no light chain restriction. The kappa and lambda ratio was approximately 1:1. Similar results were seen in the sialadenitis cases. In contrast, the lymphoma case showed a nodular and diffuse proliferation of small B-cells (CD20+) with only a minority of scattered reactive T-cells (CD3+), and with evidence of kappa light chain restriction and
**Discussion**

The histogenesis of the lymphoid stroma in Warthin’s tumor remains uncertain. Unlike in other locations of salivary glands, the parotid gland is encapsulated at late embryonal development stage. Lymph nodes developed nearby may happen to be found within the parotid gland capsule. Also salivary gland tissue may be found within these lymph nodes [18, 19]. The lymphoid stroma, which typically has numerous germinal centers, may result from a response by the immune system to the epithelium of Warthin’s tumor. Conversely, it may represent residual lymphoid tissue in lymph nodes partially replaced by ectopic, proliferative ductal epithelium [20-22]. In these background, it has been conceptualized that Warthin’s tumor is a neoplasm that develops from heterotopic salivary ducts trapped within intraparotid or periparotid lymphoid tissue. A more likely explanation is that it is a metaplastic process with a secondary lymphoid reaction. In support of this proposal is the finding that several other benign and malignant salivary gland tumors appear to incite a prominent tumor associated lymphoid proliferation [23, 24].
Eighteen cases of Warthin’s tumor associated with malignant lymphoma have been documented in the English literature (Table 1). Most of these cases were low grade B cell lymphomas, mostly of follicular lymphoma. One diffuse large cell lymphoma was noted which may arise de novo or secondary to either MALT or follicular lymphoma [25].

The relationship of NHL associated with Warthin’s tumor to previous radiation therapy was discussed [26]. In our cases, no patient had previous history of radiation therapy.

The purpose of this study was to determine the prevalence of occult B-cell monoclonals in unselected parotid gland specimens with Warthin’s tumor. Since most reported cases were low-grade follicular lymphoma, we catalogued the distribution of T and B cell markers in this tumor and searched for kappa and lambda light chain restriction by IHC. FISH and PCR methods were also used to detect the prevalence of occult IgH gene (14q32.3) and bcl-2 gene t(14;18)(q32;q21) rearrangement. IHC analysis revealed mixed T and B lymphocytic components, with a polyclonal distribution of the latter. FISH/PCR studies failed to demonstrate a t(14;18) in any of the fourteen cases under investigation. These results support the concept that the lymphoid stroma of Warthin’s tumor is benign and reactive [27].

In a recent article [28], B cell and T cell components of 20 Warthin’s tumor cases were also found polyclonal. Minor clonal expansions of both B and T cells were also noted in PCR up to 50% of tumors, which was consistent with an intense, restricted immune response in this tumor. However, any information about the antigens inciting this response was not provided. There was no evidence of bcl-2 proto-oncogene translocation. Interestingly, 95% of these cases of this article contained detectable Epstein-Barr virus DNA. The idea of the lymphoid stroma having features of MALT was debated, because lymphomatous transformation of Warthin’s tumor is extremely uncommon compared to MALT-type lymphoma in other organs [11].

Since sustained antigenic stimulation and reactive hyperplasia may precede the development of low-grade B-cell lymphoma, this mechanism could play a role in the histogenesis of the lymphoid stroma of
Lymphoid stroma and Bcl 2 gene

Conclusions
Our study indicated that the lymphoid stroma of Warthin’s tumor is reactive. Occult B-cell monoclonality as determined by IgH FISH/PCR and bcl-2 FISH/PCR were not identified in unselected cases of Warthin’s tumor and established the benign polyclonal nature of the lymphoid stroma in this neoplasm.

References


12. Saxena A, Memauni B and Hasegawa W. Initial diagnosis of small lymphocytic lymphoma in Table 1. Summary of cases of malignant lymphoma associated with Warthin’s tumor

<table>
<thead>
<tr>
<th>No.</th>
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*: Non-Hodgkin’s lymphoma; **: Hodgkin’s Disease; #: Lymph node

Warthin’s tumor and set the stage for the subsequent evolution of malignant lymphoma within the neoplasm [29].


