Introduction

The ocular adnexa comprise the surrounding structures of the eye, including the eyelids, conjunctiva, lacrimal gland and apparatus, and orbital soft tissue. The most common ocular adnexal malignancy is lymphoma, which accounts up to 55% of all orbital disease. Ocular adnexal lymphoma compromises approximately 1-2% of all non-Hodgkin lymphomas and 8% of extranodal lymphomas. Usually the ocular adnexal region is the primary site of disease with secondary involvement by systemic disease occurring in only 10-32% of cases.

Eighty to 95% of ocular adnexal lymphomas are low-grade B-cell lymphomas. The most common lymphoma is extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma), which accounts for approximately 50-55% of all disease. Other commonly encountered small B-cell lymphomas are follicular lymphoma (25%), mantle cell lymphoma (5%) and chronic lymphocytic leukemia/small lymphocytic lymphoma (5%). Diffuse large B-cell lymphoma accounts for less than 10% of cases while other high grade B-cell lymphomas, T and NK-cell lymphomas and Hodgkin lymphoma are rare, accounting for less than 1% of all cases.

Since the most frequently encountered lymphomas in the ocular adnexa are MALT lymphoma and follicular lymphoma the following discussion focuses on these two diseases entities.

Extranodal Marginal Zone Lymphoma of Mucosa-Associated Lymphoid Tissue (MALT Lymphoma)

MALT lymphoma is a disease of older adults (median age = 65 years) affecting females more often than males (M:F = 1:1.4). The ocular adnexa are the primary site of disease in approximately 90% of cases. It occurs most often in the orbital soft tissue with predominantly unilateral involvement. MALT lymphoma is thought to result from protracted lymphocyte proliferation in the setting of chronic antigenic stimulation; however, the causative antigen has not been definitively identified. There is some evidence that suggests an association with *Chlamydia psittaci* infection in at least some cases but the exact role this organism plays in ocular adnexal MALT lymphomagenesis remains unclear.
Morphologically the tumor is composed of a heterogeneous population of “centrocyte-like” lymphocytes, plasmacytoid lymphocytes, mature plasma cells, and transformed blasts that either surround reactive lymphoid follicles in a marginal zone pattern or infiltrate and disrupt lymphoid follicles imparting a nodular appearance\(^1\). The malignant B-cells have a non-specific immunophenotype and usually express pan-B-cell markers (CD20, CD79a, PAX5), surface immunoglobulin (usually IgM) and BCL2, with variable expression of CD43 and cytoplasmic immunoglobulin, and absence of CD10, BCL6, CD23 and cyclin D1. Cases with CD5 expression have been described but these are rare. Immunohistochemical stains for CD21 and CD23 highlight follicular dendritic cell meshworks within benign lymphoid follicles, which are expanded and disrupted in cases with follicle colonization. Stains for BCL6 and CD10 are useful in this setting, as they typically highlight follicular colonization and disruption of the underlying benign germinal centers.

At the genetic level, several recurrent abnormalities are seen. The two most common abnormalities are trisomy 3 and trisomy 18, seen in upwards of 68% and 57% of cases, respectively\(^1\). Trisomy 3 occurs most frequently in the orbital soft tissue whereas trisomy 18 is evenly distributed between the orbit, conjunctiva and lacrimal gland\(^1\). While these abnormalities are common, they are not specific for MALT lymphoma (see follicular lymphoma, below).

Four recurrent mutually exclusive genetic abnormalities have been identified in ocular adnexal MALT lymphoma that all converge upon the nuclear factor-κB (NF-κB) pathway: \(t(11;18), t(14;18), t(3;14),\) and \(A20\) gene inactivation\(^1\)\(^-\)\(^7\)\(^1\). A detailed description of the NF-κB pathway is beyond the scope of this discussion and the reader is referred to other sources\(^1\)\(^8\).

\(t(11;18)(q21;q21)\)

\(t(11;18)(q21;q21)\) occurs in 16% of cases\(^1\)\(^6\) and does not occur in association with trisomy 3 or 18. The translocation fuses the \(MALT1\) gene on chromosome 18q21 with the \(API2\) gene on chromosome 11q21, leading to the expression of a chimeric \(API2-MALT1\) fusion protein\(^1\)\(^9\). The chimeric protein is able to spontaneously oligomerize and bind to TRAF6, mediating TRAF6 oligomerization and downstream activation of the NF-κB pathway\(^2\)\(^0\). Furthermore, the fused \(API2\) protein loses its normal E3 ubiquitin ligase activity and cannot downregulate the expression of BCL10, an upstream effector in NF-κB activation, resulting in loss of this negative-feedback loop\(^2\)\(^1\).

\(t(14;18)(q32;q21)\)

\(t(14;18)(q32;q21)\) is detected in up to 24%\(^2\)\(^2\) of cases and is often seen in association with trisomy 3 and/or 18\(^1\)\(^4\). It juxtaposes the \(MALT1\) gene to the enhancer region of the \(IGH\) gene on chromosome 14q32 resulting in constitutive overexpression of the MALT1 protein. MALT1 plays numerous roles in NF-κB activation: it activates the canonical NF-κB pathway through synergistic BCL10 interaction\(^2\)\(^3\), it is involved in BAFF-mediated activation of the non-canonical NF-κB pathway\(^2\)\(^4\), and it is able to cleave and inactivate the NF-κB inhibitor \(A20\)\(^2\)\(^5\). Constitutive overexpression of MALT1 likely
results in increased activity in all of these pathways, which ultimately leads to uncontrolled NF-κB activity.

\[ t(3;14)(p14;q32) \]

\[ t(3;14)(p14;q32) \] is seen in 20%\(^\text{15}\) of cases and is also associated with trisomy 3 and/or 18. The translocation results in juxtaposition of the \textit{FOXP1} gene on chromosome 3p14 to the \textit{IGH} enhancer resulting in overexpression of the FOXP1 protein. The biological function of FOXP1 is largely unknown but it has been shown that overexpression of some FOXP1 isoforms is associated with increased expression of NF-κB associated genes in lymphoma\(^\text{26}\).

\textbf{A20 Inactivation}

Deletions, inactivating mutations, and promoter methylation of the \textit{A20} (\textit{TNFAIP3}) gene, located on chromosome 6q23.3, are seen in 37% of translocation-negative ocular adnexal MALT lymphoma\(^\text{17,27}\). \textit{A20} is a known inhibitor of the NF-κB pathway and lymphomas with loss of this protein exhibit increased NF-κB activity\(^\text{28}\). \textit{A20} inactivation does not occur in association with the previously discussed translocations and it is associated with poor disease-free survival\(^\text{27}\).

Two other translocations have been identified in ocular adnexal MALT lymphomas: \( t(5;14)(q34;q32) \) and \( t(9;14)(p24;q32) \).\(^\text{29}\). These translocations result in juxtaposition of the \textit{ODZ2} gene on chromosome 5q34 and the \textit{JMJD2C} gene on chromosome 9q32 to the enhancer region of \textit{IGH}, resulting in ODZ2 and JMJD2C protein overexpression. The roles these potential oncogenes play in MALT lymphomagenesis are unknown and require further investigation.

\textbf{MALT Lymphoma and the Molecular Pathology Laboratory}

RT-PCR primer sets have been developed to detect the \( t(11;18) \). The advantage of RT-PCR is that it can be performed on small tissue specimens that are not amenable to FISH analysis. It is also highly sensitive making it ideal for assessment of minimal residual disease. A commercial dual-color dual-fusion FISH probe is available for the \( t(11;18) \) and can be used as an alternative to RT-PCR. A commercial dual-color dual-fusion probe is also available for the \( t(14;18) \) (\textit{IGH-MALT1}) and there is a break-apart probe for the \textit{MALT1} gene. Commercial probes for the centromere of chromosome 3 (CEP3) and chromosome 18 (CDP18) are available which can be used to detect trisomy 3 and 18, respectively. Assays for the detection of the \( t(3;14) \) or inactivation of \textit{A20} are not currently available in the clinical molecular pathology laboratory.

At present, detection of a specific genetic abnormality in MALT lymphoma is not required as it has no impact on therapy; however, detecting these abnormalities can be useful aids in accurately diagnosing and classifying the disease. To differentiate MALT lymphoma from other lymphomas, such as follicular lymphoma, use of the \textit{MALT1} break-apart probe is recommended. To differentiate MALT lymphoma from a benign lymphoid proliferation use of the CEP3 probe is recommended (in addition to \textit{IGH} gene rearrangement studies by PCR, see below).
Follicular Lymphoma

Follicular lymphoma is a germinal center derived B-cell lymphoma and is the second most common lymphoma involving the ocular adnexa. Like MALT lymphoma it is a disease of older adults (median age 64 years) and affects females more often than males (M:F = 1:1.7)\(^8\). The orbital soft tissue is the most frequently involved site and the disease is usually primary to the ocular adnexa but approximately 30% of cases represent secondary involvement\(^8\).

Ocular adnexal follicular lymphoma is similar to its nodal counterpart, consisting of neoplastic lymphoid follicles occupied by a mixture of malignant centrocytes and centroblasts\(^11\). In most cases the tumor is low-grade and centrocytes predominate. Areas of diffuse growth can occasionally be encountered. The tumor cells typically express pan-B-cell markers (CD20, CD79a, and PAX5), surface immunoglobulin, and the germinal center-associated proteins CD10 and BCL6. In the majority of cases they aberrantly express BCL2 and lack expression of CD5, CD23, CD43 and cyclin D1. Immunohistochemical stains for CD21 and CD23 highlight intact follicular dendritic cell meshworks within neoplastic follicles.

**t(14;18)-positive follicular lymphoma**

The genetic hallmark of follicular lymphoma is the t(14;18)(q32;q21). It is present in approximately 85-90% of cases and enriched in low-grade disease, with grade 3B follicular lymphoma least likely to harbor the abnormality. The translocation juxtaposes the BCL2 gene on chromosome 18q21 to the enhancer region of the IGH, which leads to aberrant overexpression of BCL2 (note: this differs from the t(14;18)(q32;q21) of MALT lymphoma involving the MALT1 gene, which lies 5Mb centromeric to BCL2). BCL2 is an anti-apoptotic protein and its overexpression in follicular lymphoma prevents the malignant cells from undergoing apoptosis. Approximately 10% of t(14;18)-positive follicular lymphoma lack detectable expression of BCL2 by immunohistochemical analysis using the standard BCL2 antibody (clone 124); however, this is due to mutations in the BCL2 gene which alter the antibody's binding site. Use of the alternate E17 antibody identifies BCL2 protein expression in these so-called ‘pseudo-negative’ cases\(^30,31\). True t(14;18)-positive/BCL2-negative follicular lymphoma is rare.

The t(14;18) is acquired during B-cell maturation in the bone marrow where VDJ rearrangement of the IGH gene occurs. It is thought to be the initial genetic event in this disease; however, it is not sufficient for neoplastic transformation as it is also found at low levels in the circulating lymphocytes of approximately 45% of healthy individuals who never develop disease\(^32\). t(14;18)-positive follicular lymphoma acquires secondary chromosomal alterations with six or more abnormalities seen in the majority of cases. Secondary abnormalities include deletions of 1p, 6q, 10q, 13q, 17p and gains of 1q, 2p, 7, 8, 12q, X and 18q. Regarding the latter abnormality, comparative genomic hybridization and single nucleotide polymorphism (SNP) studies have shown gains/amplification of the BCL2 locus on chromosome 18q21 are restricted to the t(14;18)-positive group\(^33\).
Recently, a novel genetic abnormality has been identified in a subset of t(14;18)-positive follicular lymphoma. Next generation sequencing studies have shown that the \( EZH2 \) gene on chromosome 7p35, harbors a recurrent heterozygous mutation at codon 641 in 7-22% of all follicular lymphoma and 28% of t(14;18)-positive follicular lymphoma.\(^{34-36} \) \( EZH2 \) mutation is not identified in t(14;18)-negative follicular lymphoma.\(^{35} \) \( EZH2 \) forms part of the polycomb repressor complex which mediates gene repression via histone trimethylation. Studies have shown that this novel gene mutation results in a ‘gain-of-function’ and increases histone trimethylation activity,\(^{37} \) which is consistent with the findings of epigenetic studies that show follicular lymphoma is associated with high levels of aberrant hypermethylation of various genes, including tumor suppressor genes such as DAPK, p15, p16, and p57 and polycomb repressor target genes.\(^{38-41} \)

### t(14;18)-negative follicular lymphoma

t(14;18)-negative follicular lymphoma accounts for 10-15% of all cases. Patients tend to present with extranodal disease at a lower stage with less bone marrow involvement.\(^{42,43} \) The tumors usually have high grade cytology (often grade 3B), focal diffuse architecture,\(^{44} \) and an alternate CD10-, MUM1+, BCL2- immunophenotype.\(^{43,45} \)

Many of these t(14;18)-negative cases harbor abnormalities of \( BCL6 \) gene, the most frequent of which is the t(3;14), which places the \( BCL6 \) gene on chromosome 3q27 under control of the \( IGH \) enhancer region. This abnormality is also frequently reported in diffuse large B-cell lymphoma; however, in follicular lymphoma the t(3;14) is associated with a different \( BCL6 \) breakpoint.\(^{46} \)

Other genetic abnormalities reported in t(14;18)-negative follicular lymphoma include; trisomy,\(^{47} \) gains of chromosome 1,\(^{48} \) and deletion of chromosome 1p36 (\( TNFRSF14 \) gene).\(^{49} \) The latter abnormality is reported to be associated with a predominantly diffuse growth pattern.

MicroRNA and gene expression profiling studies have shown that t(14;18)-negative follicular lymphoma, unlike its t(14;18)-positive counterpart, exhibits a “late” germinal centre or activated B-cell-like profile with differential miRNA expression and upregulation of genes involved in NF-\( \kappa \)B signally and cell proliferation.\(^{33,50} \)

### Follicular Lymphoma and the Molecular Pathology Laboratory

A PCR based assay can be used to detect the t(14;18) (\( IGH-BCL2 \)) in FFPET. Its sensitivity is limited by the fact that there are multiple breakpoints in the \( BCL2 \) gene. Primer sets have been developed that target the most common breakpoints including: the major break point region (MBR), minor cluster region (MCR) and intermediate cluster region (ICR). When these three primer sets are used assay sensitivity is approximately 70-85%. Conversely, a commercial dual-color dual-fusion FISH probe for the t(14;18) is available, which has a superior sensitivity to PCR (100% vs 36-69%)\(^{51-53} \), with the BIOMED-2 PCR protocol demonstrating the highest sensitivity. FISH is the preferred method to detect the t(14;18) in the clinical laboratory.
At present, detection of genetic abnormalities in follicular lymphoma is not required as it has no impact on therapy; however, detecting these abnormalities can be useful aids in accurately diagnosing and classifying the disease. To differentiate follicular lymphoma from other lymphomas and reactive lymphoid proliferations the t(14;18) by FISH analysis is recommended. Break-apart probes for BCL2 and BCL6 are also available, which can also be used to identify a clonal lymphoid proliferation and help differentiate follicular lymphoma from MALT lymphoma.

**Molecular Testing for B-cell Clonality in Ocular Adnexal Lymphoid Proliferations**

Lymphoid proliferations in the ocular adnexa are not limited to malignant lymphoma and the differential diagnosis includes benign conditions such as reactive lymphoid hyperplasia, atypical lymphoid hyperplasia, and inflammatory pseudotumor. In equivocal cases demonstration of a monoclonal B-cell population by molecular testing for B-cell clonality can help make a definitive diagnosis.

Molecular diagnosis of B-cell clonality relies upon the detection of rearranged immunoglobulin genes. During B-cell maturation in the bone marrow the immunoglobulin heavy chain gene (IGH), located on chromosome 14q32, undergoes RAG1/RAG2-dependent rearrangement of its discontinuous variable (V), diversity (D), and joining (J) regions. Successful IGH rearrangement leads to rearrangement of the V and J regions of the immunoglobulin kappa light chain gene (IGK), located on chromosome 2p11. In approximately one third of maturing B-cells IGK rearrangement is unsuccessful so the immunoglobulin lambda light chain gene (IGL), located on chromosome 22q11, undergoes rearrangement. Every B-cell that matures and leaves the bone marrow to populate the peripheral lymphoid tissue has its own unique set of IGH and IGK or IGL genes with unique gene sequences of a unique length. Since lymphoma is a proliferation of monoclonal lymphocytes derived from a single transformed B-cell the detection of a single rearranged IGH and/or IGK/IGL gene within a B-cell proliferation is considered consistent with a monoclonal B-cell population and a diagnosis of lymphoma.

Polymerase chain reaction (PCR) based approaches are the preferred method for B-cell clonality testing in the clinical laboratory today. In general, sample DNA is amplified using primers directed against the V(D)J region of the immunoglobulin genes and the size of the amplified DNA products are then analyzed using polyacrylamide gel electrophoresis or fluorescence-labeled capillary gel electrophoresis. Monoclonal populations yield a DNA product of a single size, whereas polyclonal populations yield DNA products of varying sizes.

False-positive and false-negative results using PCR-based methods occur. False-positives often result when there is limited template DNA because only a few benign B-cells are present in the specimen. False-negatives are usually due to poor primer design/annealing or somatic hypermutation of the target immunoglobulin gene (SHM). SHM is a normal part of B-cell development that takes place in the germinal center where B-cell receptor stimulation results in increased activation-induced cytidine
deaminase activity, which in turn introduces point mutations into the rearranged immunoglobulin genes. Mutations introduced at primer sites may result in reduced or absent primer annealing. Since this process occurs in the germinal center false-negative PCR results due to SHM are more frequently seen in lymphomas derived from cells that have travelled through the germinal center, including marginal zone lymphoma and follicular lymphoma. To overcome this issue the BIOMED-2 Concerted Action Project BMH4-CT98-3936 has designed a standardized multiplexed PCR protocol that includes 28 \( IGH \) primers (20 V, 7 D, 1 J), 8 \( IGK \) primers (6 V, 2 J), and 3 \( IGL \) primers (2 V, 1 J)\(^{54} \). The comprehensive nature of the assay largely eliminates false-negatives due to inadequate gene coverage and SHM and substantially increases sensitivity (>95%) in detecting B-cell clonality in both marginal zone lymphoma and follicular lymphoma\(^{54-57} \).

References


Update on the Molecular Pathology of Ocular Adnexal Lymphomas

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Introduction

• Ocular Adnexa
  – Orbital soft tissue, conjunctiva, lacrimal gland and apparatus, eye lids

• Ocular Adnexa Lymphoma
  – Most common malignancy of ocular adnexa
    • 20-55% of all orbital disease
    • 1-2% of all non-Hodgkin lymphomas
    • 8% of extranodal lymphomas
  – Usually primary site of disease
    • 10-32% represent involvement by systemic disease
Introduction

- Ocular Adnexal Lymphoma
  - MALT lymphoma 50%
  - Follicular lymphoma 25%
  - Mantle Cell lymphoma 5%
  - CLL/SLL 5%
  - DLBCL 10%
  - Others <5%

- Most lymphomas are low-grade B-cell neoplasms
- MALT lymphoma and follicular lymphoma constitute the majority of ocular adnexal lymphomas
MALT Lymphoma

• Extranodal marginal zone lymphoma of mucosa-associated lymphoma tissue (MALT) lymphoma
• Most common lymphoma of ocular adnexa
• Median age = 65 years, M:F = 1:1.4
• Site of disease
  – Orbital soft tissue > conjunctiva > lacrimal gland
  – Unilateral in 90%
  – Primary in 90%
• Etiology:
  – Chronic antigenic stimulation - *Chlamydia psittaci*
  – Derived from post-germinal center B-cells
MALT Lymphoma

• **Morphology**

  – “centrocyte-like” lymphocytes, plasmacytoid lymphocytes, mature plasma cells, transformed blasts

  – **Marginal zone growth pattern** → malignant cells surround intact benign reactive follicles

  – **Nodular growth pattern** → malignant cells surround and infiltrate benign reactive follicles
MALT Lymphoma

• **Immunophenotype**
  — Positive:
    • CD20, CD79a, PAX5
    • sIg, BCL2
  — Variable:
    • CD43, cIg
  — Negative:
    • CD5, CD10, BCL6, CD23, cyclin D1

— FDC Meshworks
  • CD21, CD23 positive
  • Marginal zone pattern FDC meshworks intact
  • Nodular growth pattern FDC meshworks expanded and disrupted.
MALT Lymphoma: Molecular Pathology

• **Numerical Abnormalities**
  – Trisomy 3
  – Trisomy 18

• **Structural Abnormalities**
  – Converge upon the Nuclear Factor – κB (NF-κB) pathway
  
  – Chromosomal Translocations:
    • t(11;18), t(14;18), t(3;14)
    • t(5;14), t(9;14)

  – Deletions/Mutations
    • A20 gene
MALT Lymphoma: Molecular Pathology

• **Trisomy 3 and 18**
  – Most common genetic abnormalities in MALT lymphoma
    • Trisomy 3 $\rightarrow$ 68% of MALT lymphoma
    • Trisomy 18 $\rightarrow$ 57% of MALT lymphoma
  – Often seen in association with t(14;18) or t(3;14)
  – Not specific for MALT lymphoma
MALT Lymphoma: Molecular Pathology

• **t(11;18)(q21;q21)**
  – 16% of cases
  – Does not occur with other genetic abnormalities
  – *API2-MALT1* translocation
  – Gene fusion results in expression of an *API2-MALT1* fusion protein

  – API2 Component
    • E3 ubiquitin ligase is **NOT** present and ligase activity is lost (ligase activity responsible for BCL10 degradation)

  – MALT1 component
    • TRAF6 binding sites present
    • Caspase-like domain present (has protease activity that inactivates A20)
MALT Lymphoma: Molecular Pathology

- \( t(11;18)(q21;q21) \)
  - API2-MALT1 spontaneously oligomerizes, binds to TRAF6 which oligomerizes, and activates downstream NF-\( \kappa \)B pathway
  - API2’s lack of E3 ubiquitin ligase activity and loss of BCL10 inactivation leads to loss of negative control of the activated NF-\( \kappa \)B pathway
MALT Lymphoma: Molecular Pathology

• **t(14;18)(q32;q21)**
  – 24% of cases
  – Associated with trisomy 3 and trisomy 18
  – *IGH-MALT1* translocation
  – Places *MALT1* gene under control of the *IGH* enhancer resulting in constitutive MALT1 overexpression
  – MALT1:
    • Activates canonical NF-κB pathway through BCL10 interaction
    • Involved in BAFF-mediated activation of the non-canonical NF-κB pathway
    • Cleaves and inactivates NF-κB inhibitor A20
MALT Lymphoma: Molecular Pathology

- $t(3;14)(p14;q32)$
  - 20% of cases
  - Associated with trisomy 3 and trisomy 18
  - $FOXP1-IGH$ translocation
  - Places $FOXP1$ gene under control of the $IGH$ enhancer resulting in constitutive $FOXP1$ expression
  - The specific role of $FOXP1$ in NF-$\kappa$B signalling is not known
  - Overexpression of $FOXP1$ associated with increased NF-$\kappa$B pathway associated genes in B-cell lymphoma
MALT Lymphoma: Molecular Pathology

• **A20 Inactivation**
  – *A20* gene (*TNFAIP3*) located on chromosome 6q23.3
  – *A20* is a global regulator of NF-κB activity and has tumor suppressor activity
  – *A20* Inactivation
    • gene deletion, mutation, or promoter methylation
    • Occurs in 37% of translocation-negative MALT lymphoma (not found in cases with translocations)
  – *A20* inactivation leads to uncontrolled NF-κB activation and upregulation of NF-κB regulated genes
  – Associated with a poor disease-free survival
MALT Lymphoma: Molecular Pathology

• Molecular Pathology Laboratory
  – FISH
    • Dual-color, dual-fusion probes: t(11;18), t(14;18)
    • Dual-color, break-apart probes: MALT1
    • Centromere probes: CEP3 and CEP18
  – RT-PCR
    • t(11;18)
      – Highly sensitive assay
      – Can be used on specimens too small for FISH
      – Can be used for minimal residual disease detection
  – No commercial assays for t(3;14) or A20 inactivation
MALT Lymphoma: Molecular Pathology

- Molecular Pathology Laboratory
  - Detection of a specific translocation is not required in MALT lymphoma
  - Detection can aid in diagnosing and classifying disease
    - *MALT1* probe:
      - Differentiates MALT lymphoma from most other lymphomas, including follicular lymphoma
      - If rearranged confirms clonal nature if lymphoid proliferation
    - *CEP3* probe:
      - Trisomy 3 is not specific for MALT lymphoma but its presence does confirm the clonal nature of the lymphoid proliferation
      - Trisomy 3 is useful in distinguishing MALT lymphoma from polyclonal lymphoid proliferations (*IGH* gene rearrangement studies are the preferred method)
Follicular Lymphoma

• 2nd most common lymphoma of ocular adnexa
• Median age = 64 years, M:F = 1:1.5
• Site of disease
  – Orbital soft tissue > conjunctiva > lacrimal gland
  – Unilateral in 90%
  – Primary in 70% (Secondary 30%)
• Etiology:
  – Unknown
  – Derived from follicle (germinal) center B-cells
Follicular Lymphoma

• **Morphology**
  - Mixture of centrocytes and centroblasts
    - Grade 1-2
      → 0-15 centroblasts/hpf
    - Grade 3A/3B
      → >15 centroblasts/hpf
  - Atypical neoplastic lymphoid follicles
    - Back-to-back
    - Attenuated mantle zones
    - Loss of polarity
  - Follicular lymphoma is usually low-grade with a predominantly follicular pattern
Follicular Lymphoma

• **Immunophenotype**
  
  — **Positive:**
  • CD20, CD79a, PAX5
  • sIg, **CD10**, BCL6, **BCL2**
  
  — **Negative:**
  • CD5, CD23, CD43, cyclin D1
  
  — **FDC Meshworks**
  • CD21, CD23 positive
  • Intact within neoplastic follicles
  • Absent in diffuse areas
Follicular Lymphoma: Molecular Pathology

- **t(14;18)(q32;q21)**
  - Present in 85-90% of cases
  - **Genetic hallmark** of follicular lymphoma
  - *IGH-BCL2* translocation
  - Acquired in the bone marrow during *IGH* gene rearrangement
  - Initial genetic event but not a transforming event
    - 45% of healthy population bear this translocation in circulating lymphocytes
Follicular Lymphoma: Molecular Pathology

• \textit{t}(14;18)(q32;q21)
  – Translocation places \textit{BCL2} gene under control of the \textit{IGH} enhancer resulting in constitutive overexpression of anti-apoptosis protein \textit{BCL2}
  – 10% of cases lack \textit{BCL2} expression using standard IHC antibody (124 clone)
    • Due to mutations in the \textit{BCL2} gene that alter the antibody binding site
    • Use of alternate antibody (E17 clone) identifies \textit{BCL2} expression in the vast majority of these ‘pseudo-negative’ cases
    • True \textit{BCL2}-negative cases are rare
Follicular Lymphoma: Molecular Pathology

- **t(14;18)(q32;q21)**
  - Usually part of a complex karyotype (>5 abnormalities):
    - Loss: 1p, 6q, 10q, 13q, 17p
    - Gain: 1q, 2p, 7, 8, 12q, X, 18q
  - Gains/amplification of \(BCL2\) on 18q21 restricted to t(14;18)-positive follicular lymphoma
Follicular Lymphoma: Molecular Pathology

- **EZH2 mutation**
  - detected in 7-22% of follicular lymphoma
  - Restricted to t(14;18)-pos follicular lymphoma
    - Up to 28% of cases
  - **EZH2** located on chromosome 7p35
  - Encodes for a histone methyltransferase
    - Part of polycomb repressor complex that mediates gene repression through histone trimethylation
  - Recurrent heterozygous mutation at codon 641 imparts ‘gain-of-function’ with increased histone trimethylation activity
  - Consistent with finding that follicular lymphoma is associated with increased aberrant hypermethylation of various tumor suppressor genes and polycomb repressor target genes
Follicular Lymphoma: Molecular Pathology

- **t(14;18)** – Negative
  - Accounts for 10-15% of cases of follicular lymphoma
  - Associated with:
    - Extranodal disease, low-stage, grade 3A/3B morphology
    - CD10-, MUM1+, BCL2- immunophenotype
  - Genetically heterogeneous
    - 3q27 (BCL6) abnormalities
      - Most common abnormality
      - t(3;14) $\rightarrow$ BCL6-IGH translocation
    - 1p36 (TNFRSF14) deletions
      - Associated with diffuse growth pattern
  - miRNA and GEP studies show a ‘late’ germinal center or activated B-cell-like profile
    - Upregulation of NF-κB signalling and cell proliferation genes
Follicular Lymphoma: Molecular Pathology

• Molecular Pathology Laboratory
  – FISH
    • Dual-color, dual-fusion probes: t(14;18)
    • Dual-color, break-apart probes: BCL2, BCL6
  – PCR
    • t(14;18)
      – Sensitivity limited by multiple BCL2 breakpoints (70-85%)
        » MBR – major breakpoint region
        » MCR – minor cluster region
        » ICR – intermediate cluster region
Follicular Lymphoma: Molecular Pathology

- **Molecular Pathology Laboratory**
  - Detection of a genetic abnormality is not required for diagnosis
  - Detection can aid in diagnosing and classifying disease
    - t(14;18) probe:
      - Differentiates follicular lymphoma from reactive lymphoid proliferation
      - Distinguishes follicular lymphoma from other low-grade B-cell lymphomas
    - **BCL2** probe:
      - Distinguishes lymphoma from reactive lymphoid proliferation
      - Can distinguish follicular lymphoma from MALT lymphoma
    - **BCL6** probe:
      - useful in diagnosing follicular lymphoma when t(14;18)-negative
B-Cell Clonality

• Ocular adnexal lymphoid proliferations not limited to lymphoma:
  – reactive hyperplasia
  – atypical hyperplasia
  – inflammatory pseudotumor

• Identification of a monoclonal B-cell population can aid in the diagnosis of B-cell lymphoma
  – Cytogenetic abnormalities (FISH, PCR, karyotype)
  – Immunoglobulin gene rearrangement
B-Cell Clonality

- **Immunoglobulin Gene Rearrangement**
  - Heavy and light chain genes rearranged in the bone marrow during B-cell maturation
    - $IGH$ (14q32) $\rightarrow$ $IGK$ (2p11) $\rightarrow$ $IGL$ (22q11)
  - Successful rearrangement of a heavy and light chain gene results in a B-cell with unique immunoglobulin gene sequences of unique length
    - Monoclonal B-cell population
      - All B-cells have immunoglobulin genes of identical length
    - Polyclonal B-cell population
      - Every B-cell has immunoglobulin genes of differing lengths
B-Cell Clonality

- **Immunoglobulin Gene Rearrangement**
  - *IG* PCR
    - Utilizes primers directed against V(D)J regions of *IG* genes
      - **BIOMED-2 Protocol:**
        » 28 *IGH* primers (20 V, 7 D, 1 J)
        » 8 *IGK* primers (6 V, 2 J)
        » 3 *IGL* primers (2 V, 1 J)
    - Amplified gene products are analyzed for gene length distribution
      - Polyacrylamide gel electrophoresis
      - Fluorescence-labeled capillary gel electrophoresis
B-Cell Clonality

• **Immunoglobulin Gene Rearrangement**
  
  – *IG* PCR
  
    • False-positive:
      – Usually due to low B-cell numbers in the specimen (i.e. limited template DNA)
  
    • False-negative:
      – Due to poor primer design/annealing or *IG* gene somatic hypermutation (SHM)
        » SHM → intentional introduction of point mutations into *IG* genes; occurs in the germinal center
      – SHM common in lymphomas that derive from germinal center or post-germinal center B-cells (ex. MALT lymphoma and follicular lymphoma)
      – **BIOMED-2** protocol substantially decreases false-negatives (>95% sensitivity) in MALT lymphoma and follicular lymphoma