Updates in ophthalmic pathology

Pia R Mendoza, Hans E Grossniklaus[1]

Ophthalmic pathology has a long history and rich heritage in the field of ophthalmology. This review article highlights updates in ophthalmic pathology that have developed significantly through the years because of the efforts of committed individuals and the confluence of technology such as molecular biology and digital pathology. This is an exciting period in the history of ocular pathology, with cutting-edge techniques paving the way for new developments in diagnostics, therapeutics, and research. Collaborations between ocular oncologists and pathologists allow for improved and comprehensive patient care. Ophthalmic pathology continues to be a relevant specialty that is important in the understanding and clinical management of ocular disease, education of eye care providers, and overall advancement of the field.

Key words: Digital pathology, eye pathology, molecular pathology, ocular pathology, ophthalmic pathology

Ophthalmic pathology has a long history and rich heritage in the field of ophthalmology, dating back to when ophthalmology was first established as a medical specialty in the 17th century. Ocular pathology began with the detailed description of the gross anatomy of the human eye. It further developed with the concept of light microscopy and histology alongside the invention of the ophthalmoscope, enabling clinicopathologic correlation. Eye pathology eventually grew from a part-time interest into a subspecialty of its own, established under both ophthalmology and surgical pathology.[1,2] Through the years, ophthalmic pathology has added value to the practice of ophthalmology by structurally defining and understanding ophthalmic disease processes and helping make ophthalmology the medical and surgical specialty it is today.

Ocular pathologists utilize the standard techniques of general pathology to make clinical diagnoses from the evaluation of surgically excised tissue, ranging from gross examination to routine histopathology and special stains. Because of their extensive knowledge of the basic anatomy and physiology of the eye, ocular pathologists are also uniquely trained to apply their expertise to various fields of research. Eye pathologists have made many important research contributions leading to the development of ophthalmic devices, therapeutic drugs, and surgical techniques. For example, it was the pioneering work of an ophthalmic pathologist from India in collaboration with an ophthalmologist that introduced a protocol for *in vitro* culture and expansion of human limbal epithelial cells. They described a method that uses a small limbal biopsy specimen to establish human limbal epithelial cell cultures by a feeder-free explant culture technique, from which cells can later on be harvested.

This procedure can be adapted for both basic research and clinical applications such as limbal stem cell transplants from autologous cultured limbal epithelial cells.[3] Many ophthalmic pathologists have teaching roles in academic institutions and are recognized for their contribution to the education of ophthalmologists and eye care providers in training.

In the early 2000s, however, a series of articles were published warning that the future of ophthalmic pathology was in jeopardy.[2,4,5] In his article in 2003, Apple discussed the "demise" of ocular pathology in the United States, stating that there has been a decrease in support for diagnostic and research ophthalmic pathology laboratories. Many factors that led to changes in practice were cited, including few candidates for ophthalmic pathology training, low volume of cases, lack of fully supported faculty positions, and competition in obtaining research funding. A similar situation happened in Europe, where several ophthalmic pathology laboratories were closed down mainly due to economic pressures.[6] Many would argue that this was a temporary situation and ophthalmic pathologists recognized that there were changes that needed to be made.[1,7] Today, ophthalmic pathology continues to thrive because of the confluence of modern technology, such as molecular biology and digital imaging, and due to the efforts of passionate individuals working to advance the field.

Combination of Ophthalmic Pathology and Oncology

In 2006, the American Association of Ophthalmic Pathologists recognized the benefits of coupling ophthalmic pathology

Departments of Pathology and Laboratory Medicine and [1]Ophthalmology, Emory University School of Medicine, Atlanta, GA, USA

Correspondence to: Dr. Hans E Grossniklaus, 1365 Clifton Road NE, BT428, Atlanta, 30322 GA, USA. E-mail: ophtheg@emory.edu

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with a clinical specialty, namely, ocular oncology because there is so much overlap between the two and a number of ophthalmologists are trained in both. In 2012, the American Association of Ophthalmic Pathologists became the American Association of Ophthalmic Oncologists and Pathologists (AAOOP). That same year, the Asia Pacific Society of Ocular Oncology and Pathology was officially founded during the International Symposium of Ophthalmology meeting held in Hong Kong, with members practicing in the Asian subcontinent and along the Pacific Basin. In 2014, the first oncology and pathology subspecialty day was held at the American Academy of Ophthalmology annual meeting in Chicago, USA.[1] The Middle East Africa Club of Oncology and Ocular Pathology, formed by a group based in Saudi Arabia, also had their first meeting that same year. In Europe, the European Ophthalmic Pathology Society has contributed much to the development of ocular pathology as a distinct discipline since it was officially launched in 1962.[8] Many members are also part of or work closely with the Ophthalmic Oncology Group, a European-based independent scientific group devoted to clinical ophthalmic oncology and related basic science research. In recent years, both ocular pathology and ocular oncology have advanced as strong and independent subspecialties worldwide. The International Society of Ophthalmic Pathology and the International Society of Ocular Oncology (ISOO) are professional societies formed for the advancement of ophthalmic pathology and oncology, respectively, throughout the world. The first issue of the journal Ocular Oncology and Pathology, the official journal of the ISOO, was published in 2014.

The partnership of ocular oncology and pathology has since been very enriching for both specialties, allowing for the seamless integration of diagnostic services, therapeutics, and clinical research. Dedicated ocular oncology specialists are increasingly becoming essential members of Ophthalmology Departments, unlike previously when ocular oncology cases would be handled by retina (retinoblastoma, uveal melanoma, and other intraocular tumors) or external disease/cornea (conjunctival tumors) specialists. Progress in the field of ocular oncology and pathology has encouraged dialog and collaboration between relevant practitioners, including medical and pediatric oncologists, radiation oncologists, and other cancer specialists. This has led to standardization in diagnostic criteria, classification, staging, and treatment strategies to provide patients with optimal comprehensive care.

Molecular Pathology

Molecular pathology is a rapidly progressing field that involves the use of nucleic acid-based techniques for the diagnosis and prognostication of neoplasms, hereditary disorders, and infectious diseases. The evolution of molecular science has made it possible to recognize the presence or absence of specific sequences of nucleic acids and abnormalities within certain chromosomal regions that are characteristic of various ocular disease entities. The scope of ophthalmic pathology has been greatly expanded by developments in molecular biology. Advances in cytogenetic and molecular pathology have led to the discovery of genetic events that enrich our understanding of the mechanisms of ocular disease and have proven to be important tools for diagnostic confirmation, prognostication, molecular targeted therapy, and genetic counseling of many

ophthalmic diseases. These methods have the potential for numerous clinical applications, the discussion of which is beyond the scope of this paper. Some of the common and emerging molecular pathology techniques used in the clinical setting in ophthalmology will be briefly discussed.

Next generation sequencing (NGS), also known as massively parallel or deep sequencing, is a high throughput technique that has revolutionized DNA sequencing technology. An entire human genome can be sequenced rapidly and cost-effectively using NGS, in contrast to the previous Sanger sequencing method which is time-consuming and costly. Although NGS has mostly superseded conventional Sanger sequencing in genome research, only in recent years has it been incorporated into routine clinical practice. One example of the use of NGS is elucidating the mutational spectrum and genotype-phenotype correlations of inherited retinal dystrophies.[9] NGS-based approaches have also been developed for the detection of RB1 gene mutations in retinoblastoma.[10,11]

Soft-tissue tumors that arise from the orbit have diverse histological subtypes and overlapping clinicopathologic features. These pose significant challenges in rendering a definitive diagnosis, often requiring studies beyond routine light microscopy and immunohistochemistry. A considerable number of soft-tissue tumors are characterized by recurrent chromosomal rearrangements (most commonly translocations) that produce specific gene fusions. A host of molecular assays has been adopted into routine clinical practice for the detection of these fusion genes. Genetic approaches commonly used in clinical practice for the detection of fusion genes and/or genomic imbalances in soft-tissue tumors are polymerase chain reaction (PCR) and fluorescence *in situ* hybridization (FISH). The range of soft-tissue neoplasms includes both benign and malignant entities, and a comprehensive list with their corresponding chromosomal and molecular abnormalities can be found in the World Health Organization classification of soft-tissue and bone tumors.[12] Soft-tissue tumors that an ocular pathologist may encounter in which genetic testing plays an increasingly important role include small round cell tumors such as rhabdomyosarcoma and Ewing's sarcoma, smooth muscle tumors such as leiomyosarcoma, adipocytic lesions such as myxoid liposarcoma, fibroblastic/myofibroblastic tumors such as low-grade fibromyxoid sarcoma and solitary fibrous tumor, and tumors of uncertain differentiation such as synovial sarcoma and alveolar soft part sarcoma.[13] The two main histopathologic variants of rhabdomyosarcoma are embryonal and alveolar, which demonstrate clinical and genetic differences. In particular, most alveolar but not embryonal variants are characterized by chromosomal translocations t(2;13)(q35;q14) and t(1;13)(p36;q14), which result in the fusion transcripts PAX3/FOXO1 (FKHR) and PAX7/FOXO1 (FKHR), respectively. FISH for rearrangements for FOXO1 can be used for diagnostic confirmation. This is prognostically important since alveolar rhabdomyosarcoma is considered higher risk compared to the embryonal subtype. However, recent studies have shown that alveolar rhabdomyosarcomas that are fusion negative for PAX-FOXO1 have an outcome similar to embryonal rhabdomyosarcoma. Solitary fibrous tumor is a tumor that usually behaves in a benign fashion, but up to 10% of cases can present with local recurrences or metastasis. Recently, NAB2/STAT6 fusion gene has been identified in solitary fibrous tumors by high-throughput transcriptome sequencing.[14]

Alternatively, immunohistochemical staining for STAT6 can be used to support the diagnosis of solitary fibrous tumor.

Uveal melanoma is the most common primary intraocular malignancy in adults. Genetic testing of uveal melanoma is useful for the identification of patients at increased risk for metastasis and enhances prognostication, especially when integrated with histological and clinical data. More than 80% of uveal melanomas have mutations in the GNAQ gene or its paralog GNA11 that encodes a G-protein-coupled receptor that is involved in the RAF/MEK/ERK pathway. GNAQ/GNA11 mutations are also found in benign precursor lesions such as congenital ocular melanocytosis and are thought to be initiating events in the pathogenesis of uveal melanoma.[15] The most common chromosomal aberration in uveal melanoma is loss of chromosome 3, which correlates with high mortality. Other chromosomal abnormalities that are clinically relevant are loss on 1p, 6q, 8, and 9p as well as gain on 1q, 6p, and 8q. Many cytogenetic and molecular tests for uveal melanoma have been investigated, each with their own advantages and disadvantages. Multiplex ligation-dependent Probe Amplification (MLPA) is a variation of multiplex PCR that was validated for uveal melanoma[16] and examines 38 loci across chromosomes 1p, 3, 6, and 8. Gene expression profile (GEP) analysis on uveal melanoma specimens is also often offered to patients for prognostication. Based on GEP, two classes of tumors have been identified that differ markedly in their metastatic potential. Class I melanomas resemble melanocytes and rarely metastasize (<5%). In contrast, Class II tumors resemble primitive neuroectodermal stem cells and have a significant risk of metastasis (>90%). In addition, Class II tumors typically have major chromosomal abnormalities such as loss of chromosome 3 (monosomy 3)[17] or harbor inactivating mutations in the BAP1 (breast cancer 1-associated protein 1) gene located on chromosome 3.[18,19] A proprietary GEP assay using an array of 15 genes has been reported to be an accurate prognostic predictor of uveal melanoma metastasis[20] and is commercially available under the trade name DecisionDx-UM.[21] The analysis of the entire genome can be performed by array comparative genomic hybridization (a-CGH) to detect gains or losses of large numbers of chromosome segments. A study using a-CGH showed that uveal melanoma metastases to the liver has a "Class II" gene signature.[22] Differentially expressed miRNAs have been correlated with clinicopathologic features in uveal melanomas with monosomy/disomy 3 chromosomal aberrations. MiRNA expression was shown to be predictive of liver metastasis and survival.[23]

Retinoblastoma is the most common pediatric intraocular malignancy. Development of retinoblastoma is due to inactivation of both alleles of the retinoblastoma susceptibility gene RB1. The RB1 gene was the first tumor suppressor gene identified and cloned, the discovery of which is largely attributed to the work of ocular pathologist T. Dryja.[24,25] RB1 is recognized as a key cell cycle regulator and proven to be involved in many different types of cancers. However, it has been shown that both copies of the RB1 gene are also lost in the benign tumor retinocytoma[26] and it is now believed that additional mutations in other genes besides RB1 are required for malignant transformation into retinoblastoma.[27] The previous GEP studies of retinoblastoma compared to normal retinal tissue identified thousands of genes with increased

and decreased expression.[28,29] Functional categories of the cognate genes were cell cycle, cell death, DNA replication, recombination and repair, cellular growth and proliferation, and cellular assembly and organization. Some of the genes of interest were genes normally expressed in photoreceptor cells of the developing retina, including retina-specific transcription factors (NRL, CRX, NR2E3) and genes encoding retinal antigens (ROM, SAG, AIPL1, RPGRIP1, TULP1, and PDE6H). It has been suggested that differentially expressed genes in retinoblastoma belong mainly to DNA damage response pathways, including BRCA1, BRCA2, ATM, ATR, E2F, and CHK1 genes.[29] Chromosomal abnormalities such as 1q and 6p gain, and 16q loss in retinoblastoma suggests that genes on these chromosomes contribute to the development of the disease (for example MDM4 and KIF14 on 1q, E2F3 on 6p, CDH11, and RBL2/p30 on 16q).[27,28] A gene expression profiling study sheds light on the unresolved debate on the cell of origin of retinoblastoma and has suggested that there are two distinct subtypes of retinoblastoma: Group 1 derived from a primitive retinal progenitor cell type and clinically invasive, and Group 2 arising from more differentiated cone photoreceptor cells and less invasive.[30] Recently, a rare subset of unilateral retinoblastoma tumors has been described that lack RB1 gene mutations but instead have amplification of the MYCN oncogene. These tumors are poorly differentiated and are found in very young infants.[31] For patients with retinoblastoma, genetic testing is typically done for the detection of mutations in the RB1 gene (sequence analysis, copy number changes, splice site analysis, and promoter methylation). With these new findings, however, MYCN copy number testing is now recommended in addition to RB1 gene analysis. Translational research initiated by an ocular pathologist resulted in a promising new form of aptamer-based targeted therapy for retinoblastoma. Subramanian et al. found that the protein nucleolin is overexpressed on the surface of retinoblastoma tumor cells. They proceeded to study the effect of a nucleolin-aptamer and demonstrated a significant inhibition of tumor cell proliferation.[32]

Cytogenetics and molecular assays are widely used in the classification of lymphoproliferative disorders. Malignant lymphoma of the orbit, ocular adnexa including conjunctiva, optic nerve or chorioretina may arise primarily or as part of systemic disease. The vast majority of ocular lymphomas are non-Hodgkin B-cell lymphomas, commonly extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT), follicular lymphoma, and large B-cell lymphoma. The identification of immunoglobulin gene rearrangements indicative of clonal B cells populations is useful for the diagnosis of B-cell lymphomas when histology and immunophenotyping are equivocal. Common rearrangements involve the immunoglobulin heavy chain gene (IGH) locus on chromosome 14 and human IGK kappa light chain locus on chromosome 2.[13,33] Rearrangements of the IGH and IGK genes can be tested by PCR if malignant lymphoma is highly suspected [Fig. 1]. The high sensitivity of molecular techniques such as PCR is especially useful for ocular specimens because in many cases, the tissue samples are minute and there is limited material for ancillary testing. The most common lymphoma of the orbit and ocular adnexa is MALT lymphoma and specific chromosomal translocations have been identified: t(11;18)(q21;q21), t(1;14)(p22;q32), t(14;18)(q32;q21), and t(3;14)
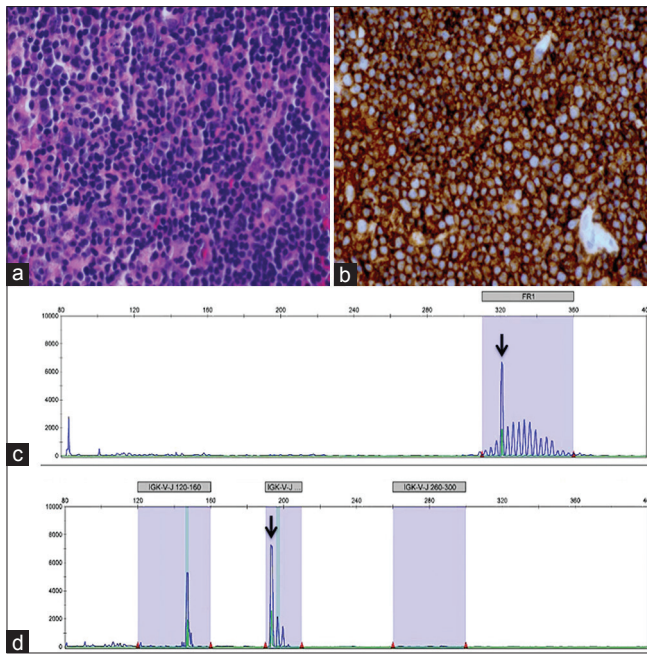
Figure 1: Polymerase chain reaction for IGH/IGK gene rearrangement in orbital marginal zone lymphoma. (a) Orbital biopsy shows an infiltrate of small, plasmacytoid, lymphocytes. (b) Immunohistochemistry for B-cell marker CD20 is positive in many cells, concerning for a B cell lymphoma. Flow cytometry was inconclusive. (c) A peak (arrow) corresponds to a polymerase chain reaction amplicon resulting from a clonal rearrangement involving the FR region of the IGH gene. (d) A peak (arrow) corresponds to a polymerase chain reaction amplicon resulting from a clonal rearrangement involving the Vk and Jk segments of the IGK gene ([a] H and E, ×150; [b] Immunoperoxidase, ×150)

(p14.1;q32). These translocations result in the production of a chimeric protein (API2-MALT1) or in transcriptional deregulation (BCL10, MALT1, and FOXP1), respectively. The lymphomas characterized by the t(11;18) translocation usually remain as low-grade lymphomas.[34] Follicular lymphoma is another common B-cell lymphoma that involves the ocular adnexa and is characterized by the chromosomal translocation t(14;18)(q32;q21), which brings BCL2 on chromosome 18 to the IGH locus on chromosome 14. This results in overexpression of BCL2 which increases the survival of tumor B-cells by inhibiting apoptosis.[35]

PCR is a powerful diagnostic tool commonly used to evaluate the anterior and posterior segment of the eye for infectious agents[36,37] (cytomegalovirus, varicella zoster virus, herpes virus, Epstein–Barr virus, toxoplasmosis, *Acanthamoeba*, chlamydia, and others) often in conjunction with cytologic analysis. Although microbial culture remains the gold standard, the technique is limited by low yield from the small volume of ocular samples.[37] Recently, PCR has been invaluable in the diagnosis and monitoring of patients infected with the Ebola virus. The latest Ebola outbreak has resulted not only in many deaths but also the largest number of Ebola virus disease survivors in history. Among survivors of Ebola virus disease, late complications that include ocular disease can develop during the convalescent phase. A case of a patient who recovered from Ebola virus disease but subsequently developed unilateral acute anterior uveitis that rapidly progressed into a panuveitis was recently reported.

Aqueous humor was aspirated from the patient's anterior chamber and tested positive for a high burden of viable Zaire Ebola virus RNA using quantitative reverse transcriptase PCR, 9 weeks after clearance of viremia. Although the pathogenesis of Ebola-associated uveitis is unknown, it was postulated that uveitis develops as a direct cytopathic effect of active viral replication persisting in an immune-privileged tissue site.[38]

Immunohistochemistry

Immunohistochemistry is a powerful laboratory technique that involves staining tissues with antibodies directed at specific antigens expressed in certain types of cells. Immunohistochemistry is frequently used as an adjunct to routine histology to diagnose and classify neoplasms. Aside from its utilization in diagnosis, immunohistochemical staining has increasing applications as a prognostic marker and guide for therapy.[39]

Immunohistochemistry has made significant contributions to the diagnosis of sebaceous carcinoma of the eyelid and ocular adnexa. The periocular region is the most frequent site for this malignancy due to the number of sebaceous glands in the eyelid, caruncle, and eyebrow. The diagnosis of sebaceous carcinoma is challenging because of the wide spectrum of clinical presentations ranging from a chalazion-like mass to local tumor spread to the conjunctiva in a pagetoid fashion. Frequent pathologic misdiagnoses in eyelid sebaceous carcinomas occur because they are misinterpreted as squamous cell carcinomas, basal cell carcinomas, or mucoepidermoid carcinomas. Among the histomorphologic features of sebaceous carcinoma is cytoplasmic vacuolization secondary to the presence of intracellular lipid. Fat stains such as Oil Red O and Sudan Black are diagnostically used however their application is limited to fresh tissue, and they are unreactive once the specimen has undergone standard tissue processing. Immunohistochemical stains for the lipid-droplet associated proteins adipophilin, perilipin, and TIP47 have recently been introduced to highlight intracellular lipid in formalin-fixed, paraffin-embedded tissue.[40] These proteins are found on the surface of intracellular lipid droplets and they function in coordinating lipid metabolism and storage. Adipophilin immunohistochemical staining has been investigated as a useful aid in distinguishing sebaceous carcinoma from other neoplasms with overlapping histology. Although adipophilin expression is observed in both sebaceous and nonsebaceous carcinomas, strong adipophilin positivity in a vesicular or vacuolar pattern [Fig. 2] is characteristic of sebaceous carcinomas with a high sensitivity (92%–100%) and specificity (85%–100%).[41-43]

In uveal melanoma, the loss of chromosome 3 and biallelic inactivating mutations in the BAP1 gene located on chromosome 3p21 encoding the BRCA1-associated protein 1 are known to be strong prognostic features of aggressive tumors and predictors of metastasis. Given the cost of BAP1 mutation analysis, immunohistochemical staining to assess BAP1 expression is a practical alternative that is more economical and gives faster results.[44,45] Negative staining for the BAP1 protein connotes lack of expression, and therefore, a likelihood of BAP1 mutation within the tumor. A study has demonstrated the strong association between BAP1 staining and BAP1 mutation status with a sensitivity
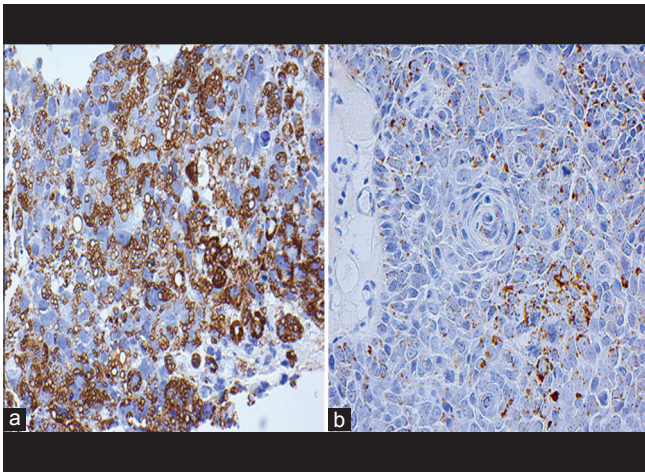
Figure 2: Adipophilin immunohistochemical staining in sebaceous carcinoma. (a) The vesicular pattern adipophilin staining is considered to be more specific for sebaceous carcinoma. (b) The granular pattern of adipophilin staining is supportive of but not specific for sebaceous carcinoma. This pattern is observed in nonsebaceous tumors and more often seen in intraepithelial/pagetoid spread of sebaceous carcinoma. ([a and b] Immunoperoxidase reaction, diaminobenzidine chromogen, hematoxylin counterstain; ×400; courtesy of Dr. Frederick Jakobiec, Massachusetts Eye and Ear Infirmary)

of 88% and specificity of 97%.[46] It has been proposed that BAP1 immunohistochemistry should be implemented in the routine histopathological examination of uveal melanoma, particularly using a red chromogen for better visualization in tumors with substantial brown melanocytic pigmentation. Immunohistochemical evaluation for BAP1 was also shown to be clinically significant as an independent predictor of death by metastasis compared to other prognostic parameters for uveal melanoma such as histologic characteristics, chromosome analysis by FISH, single nucleotide polymorphism analysis, and classification by gene expression profiling.[47]

Studies on conjunctival melanoma show similarities with cutaneous melanoma, including the presence of mutations in the BRAF proto-oncogene. BRAF is a member of the RAF kinase family of growth signal transduction protein kinases. The BRAF protein plays a role in regulating the MAP kinase/ERK signaling pathway, which affects cell division and differentiation. BRAF V600E mutations have been identified in up to 50% of conjunctival melanomas[48] and shown to be associated with a distinct clinicopathological profile, similar to BRAF-mutated cutaneous melanoma. Immunohistochemical staining can also be used to detect BRAF mutations in the primary conjunctival melanomas.[49] Approximately 50% of metastatic conjunctival melanomas respond to targeted therapy in the form of systemic BRAF inhibitors, prompting some centers to perform routine testing for the mutation.[50]

Immunohistochemistry plays a valuable role in the evaluation of lacrimal gland tumors. The use of discriminatory tumor markers pleomorphic adenoma gene 1 (PLAG1) for pleomorphic adenomas and MYB for adenoid cystic carcinomas allows the distinction between the two in cases with overlap in histologic features and unusual variants such as myoepithelioma, atypical pleomorphic adenoma, and basaloid type of adenoid cystic carcinoma. The recurrent translocation t(5;8)(p13;q12) is highly specific for pleomorphic adenoma, and results in the overexpression of the PLAG1 protein which can be detected by immunohistochemistry. The characteristic translocation in adenoid cystic carcinoma is t(6;9)(q22-23;p23-24), resulting in the loss of regulation of the oncogene MYB and high expression of the MYB protein. This also leads to overactivation of critical MYB targets that include genes involved in apoptosis and cell-cycle control.[51]

Digital Pathology

Conventional histopathology is rapidly shifting toward digitalization. Improvements in digital technology have led to the creation of glass slide scanners that are able to produce whole slide images (also called digital or virtual slides) with high resolution capture of slide details. These virtual pathology slides can be explored remotely using image viewer platforms in a way comparable to using a conventional microscope. Three-dimensional images of gross specimens can also be assembled. Digital images are used in pathology for education, diagnostics, archiving, and research.

Virtual microscopy plays an increasing role in pathology education. Glass slide boxes in medical schools are being replaced by digital slide collections; digital slide seminars and virtual microscopy are used for continuing medical education in pathology.[52] Whole slide images can be made available to multiple examiners from diverse geographical locations through the internet. Several internet-based eye pathology teaching programs have been introduced, such as the Internet-based Eye Pathology Teaching Initiative of the Emory Eye Center. An ophthalmic pathology virtual microscopy working group has been established by the AAOOP to create an Ophthalmic Pathology Collaborative and Educational Resource for ophthalmologists, pathologists, ophthalmology residents, and medical students worldwide. Additional potential benefits include Continuing Medical Education for ophthalmologists and eye pathologists, and Quality Assurance programs for practicing eye pathologists. High-quality histopathologic specimens for ophthalmic pathology from a large database are scanned using technology available from Aperio® (Leica Biosystems, Nussloch, Germany), and specimens are annotated with educational information. The virtual slides are available through the AAOOP website: Http://www.aaoop.org/learning-center/virtual-eyepath-slides/.

Still or dynamic images can be transferred by the means of network connections to be assessed by another pathologist at a remote site for teleconsultation or frozen section diagnosis instead of meeting physically or sending cases through the mail. Digital pathology can be applied to cytology screening, quality assurance, diagnostic validations for clinical trials, and quantitation of immunohistochemical stains. Histology laboratories are beginning to routinely incorporate digital image acquisition as part of their workflow. Efforts to validate whole slide imaging systems for diagnostic use are ongoing.

There are also applications of digital pathology in research, allowing more objective and automated quantitation of a variety of morphological, and immunohistological parameters.[53] Whole slide imaging was used to objectively measure nuclear morphometric characteristics in a study on the grading of anaplasia in retinoblastoma [Fig. 3].[54] Glass slides were digitally scanned, and automated image analysis software
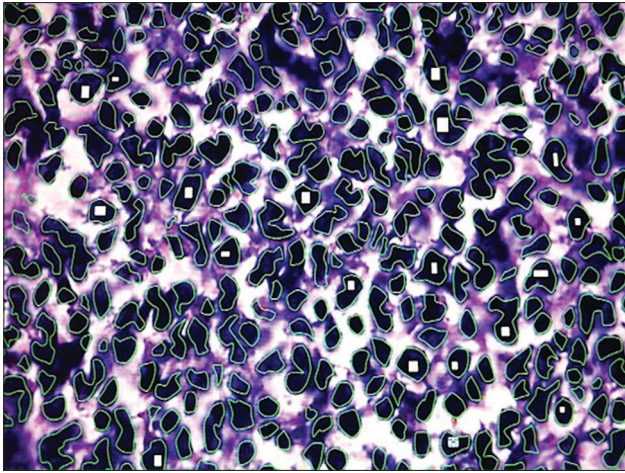
Figure 3: Whole slide imaging for nuclear morphometric analysis. Glass slides were digitally scanned (Hamamatsu Nanozoomer 2.0HT, Hamamatsu Photonics, Hamamatsu, Japan) and automated image analysis software (Matlab, Mathworks Inc., Natick, MA USA) was used to identify cell nuclei and measure nuclear size, shape, and color intensity

was used to identify cell nuclei. Based on the digital images, measurements were then made for nuclear size, shape (degree of pleomorphism), and color intensity (hyperchromicity). The study showed that in the absence of high-risk histologic features, severe anaplasia identified an additional risk for metastasis and death. Grading of anaplasia may be a useful adjunct to standard histopathologic criteria in identifying retinoblastoma patients who do not have high-risk histology but still have an increased risk for metastasis and may need adjuvant therapy.

Conclusion

Ophthalmic pathology has endured as a specialty and developed significantly through the years. The efforts of individuals committed to this field and the advent of molecular biology and digital technology have ushered us into an opportune time in the history of ocular pathology. The arrays of cutting-edge techniques available to ophthalmic pathologists are extremely powerful and pave the way for exciting new developments in diagnostics, treatment, and research. Ophthalmic pathology continues to be relevant in the present day – an area of expertise that is important in the basic understanding of eye disease, fundamental in the training of future ophthalmologists, and contributory to the overall advancement of ophthalmology.

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Conflicts of interest
There are no conflicts of interest.

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