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A Practical Approach to Understanding the Pathology of Eyelid Tumours

By David Howarth, MD

"Know thy enemy: know thyself" paraphrases Sun Tzu in *The Art of War*, an ancient treatise on the practice of warfare and the sociopolitical implications of war. Sun Tzu was an Oriental general retained by a Chinese king to help establish an army. The phrase is equally valid in the continuous clinical battles to maintain health, waged on a daily basis. Using the war paradigm, with "disease" as the enemy, medicine is steeped in this tradition with a clinical armamentarium executed by written orders. Drug regimens are issued and surgical interventions are undertaken with the hopes of eradicating the enemy.

It is in the clinical backwaters of pathology wherein one learns about one's enemy. Pathology acts as a framework upon which to extrapolate the clinical signs and symptoms of disease processes and, in turn, plan possible interventions. It is through the understanding of these disease processes that clinicians can call upon their repertoire of management protocols to fight the enemy. By knowing the behaviour of certain tumours, it is possible to draw up a battle plan to win the war. It is also possible to know which battles may be lost.

Surgical pathologists are tumour taxonomists, trained to classify and subclassify abnormal growths. At the heart of the matter is the separation of benign processes from malignant ones, with the attendant myriad of "grey" zones. It is frustrating for pathologists when they are shown cases they cannot classify. This innate ability and need to classify tis promoted from birth. Surgical pathologists are trained to look at pink and blue smudges on glass slides and establish a diagnosis that will then dictate treatment regimens. So, how do they do it? I don't think I can entirely answer "why," except to say that it is an intellectual challenge.

Benign or malignant?

The nucleus

In 1858, Rudolph Virchow established the cell as the basic unit affected by disease processes. Tumour diagnosis is based on the cytologic assessment of cellular components by evaluating the nucleus and cytoplasm. It is the nucleus that determines whether a cell is benign or malignant. Malignant nuclei usually exhibit the following criteria:

- nuclear enlargement as the result of increased DNA synthesis or chromosomal abnormalities with a subsequent increase in the nuclear to cytoplasmic ratio.
- hyperchromasia or intense nuclear staining due to increased DNA content.
- irregular nuclear contours (angulation).

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FACULTY OF MEDICINE
University of Toronto

Department of Ophthalmology and Vision Sciences

Department of Ophthalmology and Vision Sciences, Faculty of Medicine, University of Toronto,
60 Murray St.
Suite 1-003
Toronto, ON M5G 1X5

Figure 1: Squamous cell carcinoma, H&E x250.
Note the nuclear atypia and loss of polarity of the epithelium.

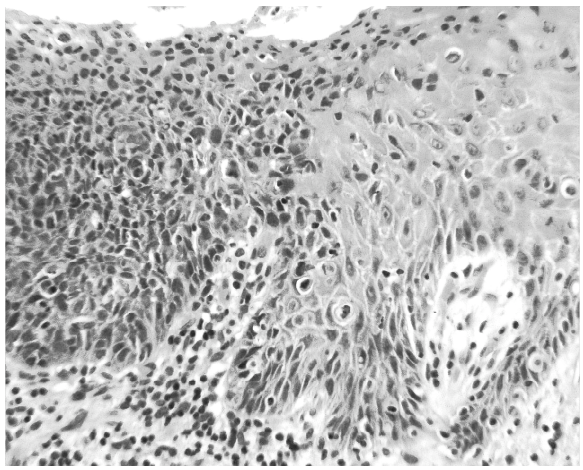
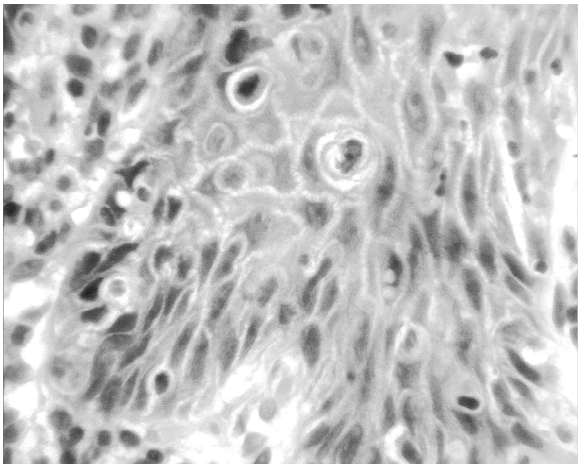


Figure 2: Squamous cell carcinoma, H&E 630.
Note the intercellular junctions (spider legs) holding the cells tightly together, thus creating a diffusion barrier.



- nuclear molding where one nucleus compresses another, indicating a fragile nuclear membrane and a lack of respect for normal cytoplasmic borders due to uncontrolled growth.

It is fortuitous that these changes occur or, as pathologists, we would be looking for other work. Mitotic activity in and of itself does not indicate malignant change (with the exception of some tumours such as leiomyosarcomas), as many reactive processes can be very mitotically active. However, atypical mitoses such as tripolar mitoses (the classic Mercedes-Benz star), with chromatids heading off in more than two directions, are virtually diagnostic of malignancy. These nuclear features of malignant tumours are often combined with an increase in cellularity and lead to the often heard expression,"blue is bad" when examining hematoxylin and eosin (H&E) stained slides. It should be noted that the ugliest-looking tumour cells are not the ones that are likely to spread or metastasize and kill the patient because their DNA complement is so discombobulated that they usually cannot replicate. As is often the case in life, it is the quiet, unassuming background tumour cells that have the potential to go "berserk" and wreak havoc.

The cytoplasm

If the nucleus determines whether the tumour is benign or malignant, it is the cytoplasm that gives the tumour its name. The phenotypic expression of the cell can be assessed by histochemistry, immunohistochemistry, and electron microscopy. Architectural features of the tumour can also be assessed by these means. Surgical pathology is not rocket science and pathologists simply name tumours after what they look like. (A similar method is used in astronomy where some constellations, star groupings, galaxies, and nebulae are named after whatever object they bear a close resemblance to). As a medical student, I remember memorizing long lists of site-specific tumours with very little clue as to how these polysyllabic tumours had derived their names. Most tumour names merely reflect their phenotypic expression. There are, of course, exceptions (eg, Schwannoma or Warthin's tumour) which do not resemble their namesakes (although faces have been seen on Mars, potatoes, potato chips, clouds, etc.). In order to achieve a modicum of immortality, some disease processes are named after their descriptors, although most of the "good" horrible diseases have already been named. I once worked with a neurosurgical resident who spent a summer doing research on pond scum and had one of the bacterial components named after him.

Tumour 1: Squamous cell carcinoma (Figures 1 and 2)

At its most basic level, the ubiquitous squamous cell carcinoma is a malignant tumour that forms intercellular bridges and produces keratin. This is all the information the pathologist needs to make a diagnosis. The nucleus has malignant criteria and the cytoplasm is eosinophilic or vacuo-

lated on an H&E stained slide. On pathological examination, vacuoles in cell cytoplasm represent 1 of 4 materials: glycogen, mucin, fat, or artifact.

Artifact is usually easy to exclude since tissue is distorted, degenerated, or poorly fixed when it is present. The other 3 materials require further histochemical assessment to sort out. A periodic acid-Schiff stain (PAS) with or without diastase (salivary amylase), Alcian blue stain, or mucicarmine stain can identify the presence of mucin, which usually renders a diagnosis of adenocarcinoma in tumour cells. PAS alone identifies both mucin and glycogen. When diastase is added to the slide, glycogen is "digested," leaving only mucin if present. Until several years ago, technologists applied diastase to glass slides by simply "spitting" on the slides being processed. For sanitary reasons, but mainly because each individual's salivary amylase levels vary, this method is no longer used and a commercial preparation of amylase is applied (This is derived from saliva and is much more expensive). Glycogen can be found in squamous cell carcinomas, but not mucin. If mucin is found in tumour cells with squamous differentiation, the tumour is classified as a mucoepidermoid carcinoma (from mucin + epidermoid, ie, squamoid).

The presence of fat in malignant tumours is another thing entirely and indicates a diagnosis of sebaceous carcinoma or liposarcoma. Fat normally dissolves in the processing of pathology slides, thereby leaving clear vacuoles. In order to demonstrate fat, special precautions must be taken in the preparation of histologic sections. By freezing fresh material and performing stains such as Oil-red-O or Sudan black, fat globules can be demonstrated in cells. Conversely, by exclusion of glycogen, mucin, and artifact, the presence of fat can be inferred on routinely processed tissues. Electron microscopy can also be used to demonstrate the presence of fat globules in cells. Electron microscopy is also useful for identifying intercellular bridges in carcinomas and intermediate filaments such as keratin (tonofilaments), neurosecretory granules, dense bodies, and a myriad of other ultrastructural features within cytoplasm.

Normal squamous epithelium has well-developed intercellular junctions with desmosomes and hemidesmosomes that form an impermeable barrier to the outside world. The formation of these intercellular junctions in a squamous cell carcinoma presents a diffusion barrier to nutrients and, therefore, squamous cell carcinomas often become necrotic as they enlarge.

Architectural growth of tumours

Assessing the architectural growth of a tumour at the time of frozen section is the primary way a pathologist makes a diagnosis. Tumour cells try to recapitulate the normal growth patterns of the cells that they differentiate towards. Better differentiated tumours re-establish this normal growth pattern very closely, while poorly differentiated tumours bear little resemblance to their namesakes, with poorly formed masses of tumour cells.

The architectural growth of tumours is the basis for histologic grading of tumours and usually correlates with biologic behaviour such as local aggressivity, metastatic potential, and prognosis. At frozen section, ice crystal formation in cells causes enlargement and angulation of the nucleus, making the assessment of nuclear criteria for malignancy much more difficult. For this reason, definitive assessment of resection margins of malignant melanomas at frozen section is not possible because the nuclear changes observed in malignant melanocytes with lentiginous spread cannot be readily identified. The best advice to the surgeon is to excise all of the pigmented lesion and, if possible, include nonpigmented margins as widely as possible. The distortion caused by freezing does not entirely revert with thawing and antigens in the cytoplasm may be compromised, thus rendering the tissue to be nondiagnostic.

Normal squamous epithelium exhibits a maturation sequence from the basal epithelial layer upwards. In dysplastic squamous epithelium, this sequence is lost by varying degrees until the point of squamous cell carcinoma in situ is reached. In squamous cell carcinoma in situ, the polarity is lost and it is difficult to tell the top from the bottom. Severe dysplasia will show minimal surface differentiation and should be treated as squamous cell carcinoma in situ (pre-invasive). Squamous cell carcinomas form mass lesions.

Tumour 2: Basal cell carcinoma (Figures 3 - 6)

Basal cell carcinoma (epithelioma) is a variant of squamous cell carcinoma, in which the basal

Figure 3: Basal cell carcinoma, H&E x 100. Note the origin from the basal epithelial layer and the artifactual separation of tumour from stroma.

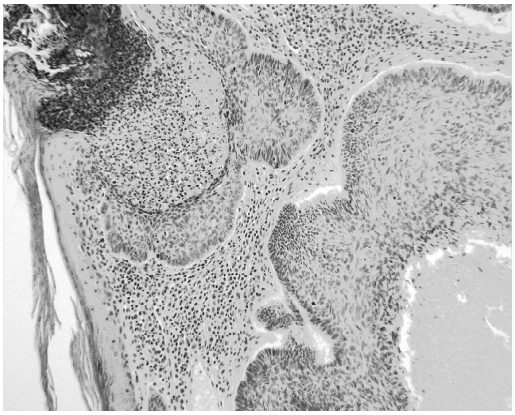
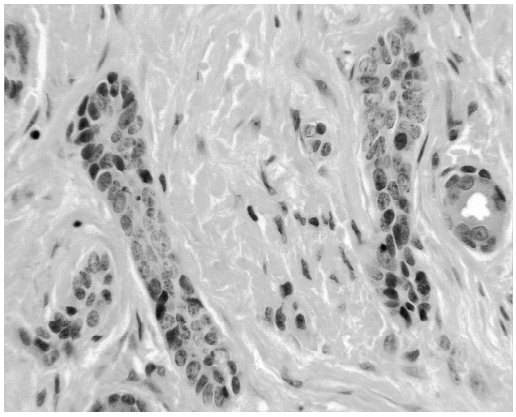


Figure 5: Desmoplastic basal cell carcinoma, H&E x 400. Note the basal palisade maintained in the invading nests that incite fibrosis (sclerosis).



epithelial layer of differentiation is maintained (basal palisade) in the tumour, with a central area containing solid, cystic, or adenoid (gland-like) growth. There is often "artifactual" separation of tumour from the adjacent dermis due to infiltration in this tissue (Figure 3 and 4). This helps in part to distinguish the tumour from adjacent tightly-bound adnexal structures, such as hair follicles that live in harmony with, rather than destroying their neighbouring surroundings. Basal cell carcinoma is considered a "Gestalt" diagnosis in pathology and it becomes a more complex topic only when explaining it to residents. Basal cell carcinomas have numerous growth patterns, including nodular, ulcerative (the classic "rodent ulcer"), desmoplastic (sclerosing or morphea) (Figure 5), and multicentric.

It is the desmoplastic and multicentric growth patterns that mandate the use of *en face* resection margins (if possible) at the time of frozen section. This enables assessment of extensive dermal infiltration under uninvolved epidermis accompanying the desmoplastic subtype or the microscopic foci of early multicentric sites. The epidermal focus in a desmoplastic basal cell carcinoma is like the "tip of the iceberg" and may not indicate the extensive sclerosing component that lies beneath. As the name implies, the tumour incites fibrosis (sclerosis, desmoplasia) in the underlying dermis. Basal cell carcinomas are locally aggressive tumours, but they rarely

Figure 4: Basal cell carcinoma, H&E x 250. Note the basal palisade of tumour cells and the artifactual retraction from the dermis.

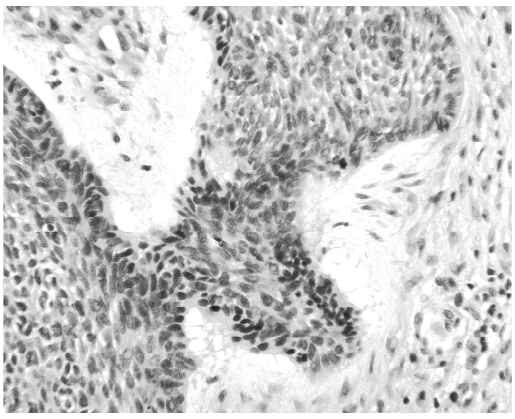


Figure 6: Basosquamous cell carcinoma, H&E x 250. Note the basal palisading of cells in one nest of invasive tumour and the lack of it in the other (squamous) nest.

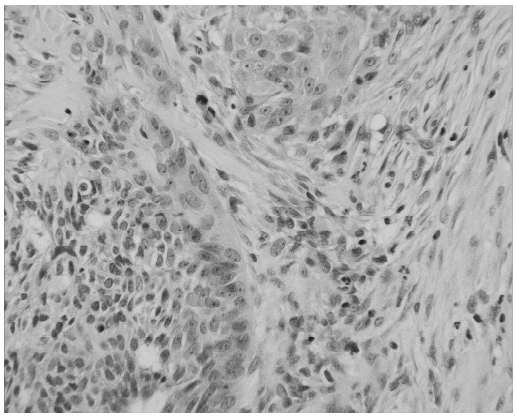


Figure 7: Sebaceous carcinoma, H&E x 250.
Note the vacuolated (fat-containing) cytoplasm.

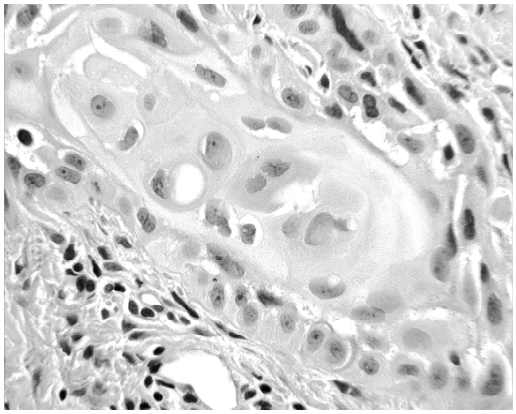
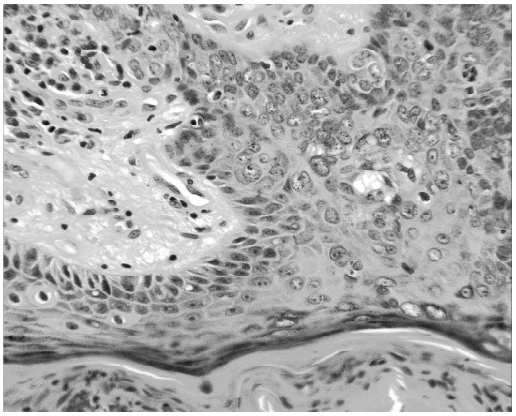


Figure 8: Pagetoid (intraepithelial) spread of sebaceous carcinoma, H&E x 250.
Note the malignant vacuolated cells present individually in the squamous epithelium.



metastasize, whereas squamous cell carcinomas are locally destructive and metastasize.

A variant known as basosquamous cell (metatypical) carcinoma should be mentioned (Figure 6). As the name implies, it is a hybrid tumour with features that are intermediate between those of a basal cell carcinoma and a squamous cell carcinoma. The tumour has regions in which the basal palisade is focally lost, leaving tumour with only the features of a squamous cell carcinoma. This tumour is locally aggressive and more likely to metastasize than the usual basal cell carcinoma (ie, it has a greater propensity to behave like a squamous cell carcinoma).

Tumour 3: Sebaceous carcinoma (Figures 7 and 8)

Sebaceous carcinomas outside of the eyelids are very rare tumours. They can present with symptoms of a chronic chalazion due to obstruction of the meibomian glands. The degree of differentiation is judged by their ability to recapitulate normal sebaceous glands. The tumour cells form lobules with a "pseudogermanitive" layer on the outer rim. The lobules can undergo central "comedonecrosis" as they mimic the holocrine secretion (whole cell) of sebaceous glands. They can spread by pagetoid invasion (intraepithelial) of the conjunctiva and squamous epithelium (Figure 8), making local control by surgical means exceedingly difficult since individual tumour cells are left behind in grossly unremarkable epithelium. At frozen section, it may be very difficult to assess resection margins due to pagetoid spread.

Sebaceous carcinomas can have extensive squamous metaplasia and mimic a squamous cell carcinoma. However, the rules state that squamous cell carcinomas cannot contain fat which, if demonstrated, establishes the diagnosis of sebaceous carcinoma. Sebaceous carcinoma can extensively infiltrate the lids, but usually does not invade the globe and can metastasize to lymph nodes. Hematogenous spread is also possible with metastases to lungs, liver, brain, and skull.

Immunohistochemistry

In the past 25 years, immunohistochemistry has become an important adjunct in the pathologic diagnosis of tumours. In immunohistochemistry, various antibodies are directed against antigens produced in cell cytoplasm or on its surface. The expression of these antigens can then be demonstrated with the use of chromagens (red or brown stains). Some of the many antigens that can be targeted in order to reveal the phenotype of the tumour in question include:

- keratins (epithelial)
- vimentin (mesenchymal)
- S-100 protein (neural crest)
- CD45 or leukocyte common antigen (lymphoid).

Genetics

As research continues to advance, the assessment of genetic abnormalities (translocations) associated with certain tumour subtypes is becoming more commonplace. Determination of these abnormalities is used as an adjunct in diagnosis and, in some cases, (eg, C-Kit is a mutation seen in malignant gastrointestinal stromal tumours that favourably predicts the response to imatinib mesylate), to establish the possible efficacy of specific drug regimens in treatment. This is a burgeoning field that will slowly transform cancer chemotherapeutic protocols and make them more specific to the individual patient's tumour.

Conclusion

The many textbooks describing pathology and ophthalmic pathology can provide more extensive detail on the above-mentioned tumours. However, I have tried to impart a greater understanding of how these diagnoses are made by giving you a surgical pathologist's perspective on these tumours. Hopefully, this practical approach will be useful to ophthalmologists who diagnose and treat patients with eyelid tumours.

Many thanks to Isabelle Schell for deciphering these cryptic notes scrawled while on the GO train.

***Dr. David Howarth** is currently an Anatomic Surgical Pathologist at Mount Sinai Hospital in Toronto, Ontario.*

Selected references

1. Huang JH. *Sun Tzu. The New Translation, The Art of War, Research and Reinterpretation.* New York: Quill, William Morrow;1993.
2. *Pathologic Basis of Disease.* 2nd ed. SL Robbins, RS Cotran, eds. W. B. Saunders;1979:1.
3. *The Lacrimal System.* JJ Hurwitz, ed. Lippincott-Raven; 1996:187-191.
4. Ramzy I. *Clinical Cytopathology and Aspiration Biopsy.* Fundamental Principles and Practice. Appleton & Lange;1990:1-25.
5. Spencer WH. *Ophthalmic Pathology: An Atlas and Textbook*, 4th ed. W.B. Saunders;1996:2249-2263, 2283-2297.

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Upcoming events

September 1-3, 2005	**Conference: 5th Annual International Society for Genetic Eye Disease** and **12th Annual International Retinoblastoma Symposium** Vancouver, B.C. Contact: 604-822-7301 or www.eyetumors.com
September 3-5, 2005	**12th Annual International Congress of Ocular Oncology** Vancouver, B.C. Contact: 604-822-7301 or www.eyetumors.com
September 29, 2005	**VPP** – "International Ophthalmology" Dr. Brian Leonard, Ottawa, Ontario
October 13, 2005	**World Sight Day**
October 15-18, 2005	**American Academy**
October 20, 2005	**VPP** – "Ethics" (TBA)
October 27, 2005	**VPP** – "Uveitis" (TBA)
November 3, 2005	**Faculty Research Day**, Vaughan Estates (5:30-7:30pm)
November 10, 2005	**VPP** – "Nutrition Supplement for AMD" Dr. Emily Chew, Bethesda, Maryland
November 17, 2005	**VPP** – "Cornea and External Diseases" Dr. Jayne Weiss, Detroit, Michigan
December 2-3, 2005	Walter Wright Day The Old Mill, Toronto, Ontario "Eye Care: What Works? What Doesn't?" Contact: Jan Spencer (416)978-1617
December 8, 2005	**VPP** – "Cornea or Plastics" (TBA)
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The Hospital for Sick Children
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