Brian Eyden • S. Sankar Banerjee
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The Ultrastructure of Human Tumours

Applications in Diagnosis and Research

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Preface

Electron microscopy is one of the great techniques developed in the twentieth century with applications in the biological sciences, and it has played its part in revolutionising the way we understand the structure and function of cells and tissues. Following its development in the 1930s, electron microscopy was applied to biological specimens, including human tissues modified by disease, initially for the purposes of research. In the 1960s, however, it became clear that electron microscopy could also have diagnostic value in medicine, since it was found that particular diseases had characteristic ultrastructural features. Applications were found in renal, neuromuscular, storage and ciliary diseases, infectious microorganisms, and tumours, to mention perhaps the most important areas. With regard to tumours, it was found that distinctive cell organelles were identifiable which could help tumour pathologists assess cell differentiation more accurately and lead to more secure diagnoses. From the 1960s to 1980s, electron microscopy was a major ancillary technique in the diagnosis of human tumours, following the classical technique of histological sections stained in haematoxylin and eosin and special stains.

The 1980s saw a significant change in the way pathologists approached the diagnosis of tumours, principally through the introduction at this time of the technique of immunohistochemistry (IHC). The use of labelled antibodies believed to be cell-lineage specific became a convenient and preferred technique, and electron microscopy suffered a significant loss of importance, with re-allocation of funding towards IHC and the closure of many clinical electron microscopy units. However, it has become clear that there are very few completely specific antibodies, a limitation of IHC requiring compensation in the form of sometimes large (and expensive) panels of immunostains in order to secure confident interpretation and diagnosis. Other limitations of IHC have become apparent – the minimal immunoreactivity of certain neoplasms, especially those showing fibroblastic differentiation; the obtaining of different results for the same antibody in different laboratories; the difficulty in sometimes distinguishing weak-positive from negative staining; and the differences in results due to changing instrumentation and technique. As a result, tumours are still encountered where there is interpretational uncertainty, and here, electron microscopy can provide additional information, which can be useful for reaching a more accurate final diagnosis, on which, after all, appropriate treatment of the patient depends.

In the belief that electron microscopy is still useful for pathologists who need precise diagnoses for the tumours they encounter in their day-to-day routine practice, as well as for scientists and doctors engaged in cancer research on *in vivo* and *in vitro* material (such as cancer cell lines and tumours in laboratory animals), we have written this atlas of tumour ultrastructure. The essential feature and objective is to add our own direct experience – our own observations and interpretations – to the existing literature. Certainly, some of our observations will be familiar to the ultrastructurally oriented reader, and these observations will constitute a confirmation of existing data – a not unimportant aspect of scientific work. More significantly, however, our book also includes new images of familiar tumours, as well as images of newly described variants – all providing novel perspectives on tumour cell differentiation. Wherever possible, we have also incorporated images of normal cells to provide information on the 'normal cellular counterpart' of tumours, which we believe is important for understanding tumour cell differentiation. In addition, our ultrastructural images are presented in the context of classical tumour pathology, with brief introductory comments on histology, immunophenotype and differential diagnosis. Rather than being a comprehensive bible of named tumours, our work is aimed at emphasising the core ultrastructural features defining a given tumour cell differentiation, as a passport through the seemingly infinite complex territory of tumour pathology and ultrastructure – in short, we want to put across the grammar of ultrastructural pathology applied to tumours, rather than a comprehensive vocabulary.

Because the book is based on our own micrographic data, there is some selectivity in the material illustrated. For tumours outside our own direct experience, our friendly colleagues have supplied some images, but otherwise, we have resorted to citing the published literature. Here again, the enormous literature on the ultrastructure of human tumours – as many as 36,000 papers according to a current Pubmed search – has forced us to be selective. Certainly, we have found it sensible to mention publications from the more accessible journals – the American Journal of Surgical Pathology, Human Pathology, Histopathology and Virchows Archiv – and understandably we have also relied particularly on the specialised journal, Ultrastructural Pathology. Although not an easy task, we have also tried to balance the older and the more recent literature. The early literature often gives more extensive ultrastructural descriptions, but can harbour out-dated terminology and concepts: by contrast, the more recent literature is more likely to offer a more meaningful immunohistochemical context, inevitably absent from the earlier ultrastructural papers, which in any case are mentioned in pre-existing monographs. In short, we extend our apologies to the authors of the many papers deserving of mention that we have not found it possible to cite.

There is so much that we still do not understand about tumour cell differentiation – about the nature and function of the structures we see during our routine diagnostic practice. Along with other exponents of electron microscopy (to be found among the references), we hope that the book will inspire investigators to study the biology of human tumours by methods which will include electron microscopy, to produce results which will enhance our understanding of tumour cell differentiation, which, in turn, might impact positively not only on diagnostic precision but also understanding the mechanism of cancer with consequences for developing therapeutic strategies.

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February, 2013

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Brian Eyden
Manchester, UK
February, 2013

Abbreviations

ACTH	adrenocorticotropic hormone
AFX	atypical fibroxanthoma
AGG	angioganglioglioma
AIDS	acquired immunodeficiency syndrome
ALCL	anaplastic large-cell lymphoma
ALL	acute lymphoblastic leukaemia
AML	acute myeloid leukaemia
APL	acute promyelocytic leukaemia
ASPS	alveolar soft-part sarcoma
ATCLL	adult T-cell leukaemia/lymphoma
AT/RT	atypical teratoid/rhabdoid tumour
AVM	arteriovenous malformation
BCC	basal-cell carcinoma
CADASIL	cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy
CE	chloroacetate esterase
CEL	chronic eosinophilic leukaemia
CLL	chronic lymphocytic leukaemia
CML	chronic myelogenous leukaemia
CNS	central nervous system
CPC	choroid plexus carcinoma
CPP	choroid plexus papilloma
DCAI	desmoplastic cerebral astrocytoma of infancy
DFSP	dermatofibrosarcoma protuberans
DIA	desmoplastic infantile astrocytoma
DIG	desmoplastic infantile ganglioglioma
DLBCL	diffuse large B-cell lymphoma
DMM	desmoplastic malignant melanoma
DMS	demarcation membrane system
DNM	desmoplastic neurotropic melanoma
DNT	dysembryoplastic neuroepithelial tumour
DRC	dendritic reticulum cell
EHD	eosinophilic hyaline droplet
EMA	epithelial membrane antigen
FAB	French, American and British
FEAM	focus of extracellular amorphous matrix
GANT	gastrointestinal autonomic nerve tumour
GCA	granular-cell astrocytoma
GCG	granular-cell glioblastoma
GCT	giant-cell tumour
GFAP	glial fibrillary acidic protein
GI	gastrointestinal
GIST	gastrointestinal stromal tumour
H&E	haematoxylin and eosin
HCL	hairy cell leukaemia
HL	Hodgkin Lymphoma
HIV	human immunodeficiency virus
HMB-45	human melanoma black 45

ICC	interstitial cell of Cajal
Ig	immunoglobulin
IHC	immunohistochemistry
IMS (IMT)	inflammatory myofibroblastic sarcoma (tumour)
LC	Langerhans cell
LCG	Langerhans cell granule
L-DOPA	L-3,4-dihydroxyphenylalanine
LGFMS	low-grade fibromyxoid sarcoma
LGM	low-grade myofibrosarcoma
MAG	monomorphous angiocentric glioma
MBP	myelin basic protein
MFH	malignant fibrous histiocytoma
MGB	Michaelis-Gutmann body
MGC	multinucleated giant cell
ML	malignant lymphoma
MNTI	melanotic neuroectodermal tumour of infancy
MPNST	malignant peripheral nerve sheath tumour
MPO	myeloperoxidase
MTS	membrane tubule system
MVB	multivesicular body
NAS-DAE	naphthol AS-D acetate esterase
NMM	neurotropic malignant melanoma
NOS	not otherwise specified
NSE	neuron specific enolase
NSE	non-specific esterase
OLC	oligodendrocyte-like cell, oligodendroglial-like cell
PA	pilocytic astrocytoma
PAS	periodic acid-Schiff
PB	pineoblastoma
PC	pineocytoma
PEC	perivascular epithelioid cell
PEComa	perivascular epithelioid cell tumour
PEL	pleural effusion lymphoma
PGNT	papillary glioneuronal tumour
PHAT	pleomorphic hyalinising angiectatic tumour
PLL	prolymphocytic leukaemia
PMA	pilomyxoid astrocytoma
PNET	peripheral primitive neuroectodermal (neuroepithelial) tumour
PNST	peripheral nerve-sheath tumour
POX	peroxidase
PPO	platelet peroxidase
PPT	pineal parenchymal tumour
PPTID	pineal parenchymal tumour of intermediate differentiation
PXA	pleomorphic xanthoastrocytoma
rER	rough endoplasmic reticulum
RGB	round granular body
RGNT	rosette-forming glioneuronal tumour
RS	Reed-Sternberg
SCC	squamous-cell carcinoma
SCAI	superficial cerebral astrocytoma of infancy
SBB	Sudan Black B
SEGA	subependymal giant-cell astrocytoma
sER	smooth endoplasmic reticulum
SFT	solitary fibrous tumour
SMA	α-smooth-muscle actin
SRC	signet-ring cell
VCR	vesicle-crowned rodlet
vs	versus
WHO	World Health Organisation
WPB	Weibel-Palade body
YST	yolk sac tumour

Contents

1

Introduction

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1.1 Introductory Remarks

Electron microscopy is one of the great scientific techniques developed in the 20th century for the study of biological specimens. After the construction of the first electron microscope in the 1930s and subsequent refinement of design, the electron microscope was applied in the field of biology from the 1950s onwards: between the 1960s and 1980s, in particular, it revealed cells and tissues in all their astonishing structural detail, and this structure formed one of the foundations for revolutionising our understanding of cell function. In addition to new research discoveries, electron microscopy was found to be useful clinically. Almost all human diseases, where classical light microscope histopathology had been important for diagnosis and understanding their nature, provided examples where electron microscopy could promote diagnostic precision and advance this understanding. The main examples include: renal disease, neuromuscular disease, tumours, infectious microorganisms (viruses, bacteria, protozoa, fungi), immotile ciliary disease, spermatozoan abnormalities and centriolopathy, metabolic storage diseases, industrial diseases (for example, asbestosis), skin diseases (CADASIL [cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy], Ehlers-Danlos syndrome, bullous diseases) and haematology (anaemias and leukaemias).

1.2 Electron Microscopy Applied to Tumours

In the electron microscopic diagnosis of non-neoplastic disease, the emphasis is usually on identifying structural features within a known tissue, cell or organelle, which define or indicate a named pathological entity. This may be the basement membrane (basal lamina) in thin-membrane disease in glomerular pathology, the presence of abnormal Z-disks in nemaline myopathy, the abundant glycogen in Pompe's disease, or the absence of dynein arms in primary ciliary dyskinesia. The cellular differentiation in these examples is mostly known in advance, whereas in the diagnosis of tumours by electron microscopy the emphasis is rather more on determining cellular differentiation: while not the only one, this is a key factor for defining and contributing to tumour diagnosis and nomenclature.

The success of electron microscopy in helping to define tumour cell differentiation (and promoting diagnostic precision) is based on the fact that each cell has a characteristic ultrastructure by which it can be identified. This ultrastructure consists of distinctive and sometimes specific structures ("organelles"), and these are "retained" in the tumoral counterpart, even, sometimes, in poorly differentiated tumours. As an example, epidermal keratinocytes are characterised by tonofilaments, desmosomes and a basal lamina, and these cell structures, readily identified by electron microscopy, can also be seen in squamous-cell carcinoma. By this means, one would be able to distinguish, for example, a carcinoma from a lymphoma (Menasce and Eyden, 2005) or a spindle-cell melanoma from a sarcoma (Zelger *et al.*, 1997). See Table 1.1 for a more comprehensive list of the cell structures and organelles which characterise the main cell types and their tumours.

Table 1.1 Main cell structures defining differentiation in common tumours

Organelle	Differentiation
Round/epithelioid cell tumours	
Rough endoplasmic reticulum (rER)	Plasma cell
Melanosomes	Melanocytic
Neuroendocrine granules	Neuroendocrine/neuronal
Mucigen granules	Glandular epithelial
Desmosomes	Epithelial
Tonofibrils	Epithelial
Basal lamina	Epithelial
Lumina and microvilli	Glandular epithelial
Long, slender, smooth microvilli	Mesothelial
Cilia	Epithelial, ependymal
Mitochondria	Oncocytic (glandular epithelial) Steroidogenic[a]
Sarcomeres	Rhabdomyoblastic
Lysosomes	Histiocytic (macrophage)
Langerhans cell ('Birbeck') granules	Langerhans cell
Glycogen	Non-specific but distinctive for Ewing's Sarcoma
Lipid	Adipocytic Steroidogenic
Smooth endoplasmic reticulum (sER)	Steroidogenic
Cell processes	Glandular epithelial Mesothelial Histiocytic
Spindle cell tumours	
Desmosomes	Epithelial
Tonofibrils	Epithelial
Basal lamina[b,c]	Epithelial, Schwannian, Perineurial, Myogenic, Endothelial ("vascular")
Processes coated in lamina	Schwannian Perineurial cell
rER	Fibroblastic Myofibroblastic Osteoblastic Chondroblastic
Smooth-muscle myofilaments with focal densities[d]	Smooth-muscle (for example, leiomyoma and leiomyosarcoma) Myofibroblastic Pericytic
Fibronexus junctions	Myofibroblastic

Notes [a] with tubular cristae.

[b] In spindle-cell tumours, other than carcinoma (for example, in Schwannian tumours or leiomyosarcoma), the term "basal" would be omitted since often the tumour cells have no identifiable apical-basal polarity to justify use of the term "basal". In these cells, it is best to use 'external lamina' or just 'lamina'

[c] Desmosomes, tonofibrils and basal lamina are also indicative of myoepithelial differentiation (which co-expresses smooth-muscle myofilaments), tumours of which may have a cell morphology intermediate between round/epithelioid and spindled.

[d] Smooth-muscle myofilaments in modestly developed amounts are common is a wide range of cell-types and tumours (Eyden, 2000).

Lymphocytes and many lymphomas have the blandest of cytoplasms – one or two mitochondria or cisternae of rER, and no junctions or cell surface structures to speak of (see Chapter 5, Table 1.1)

1.3 Impact and Limitations of Immunohistochemistry

Electron microscopy is far from being the only technique for defining cellular differentiation, and in contemporary pathology, immunohistochemistry (IHC) is one of the most important ancillary techniques for providing this kind of information. IHC was originally based on identifying cell-specific or tumour-specific marker-proteins, and the convenience and early belief in antibody specificity impacted adversely on the practice of electron microscopy. Many clinical centres experienced a significant re-orientation of resources towards IHC at the expense of electron microscopy, and, as a result, in the 1980s, many small electron microscopy units closed down.

However, as the technique of IHC matured, it became clear that there are very few completely specific antibodies, a limitation it is sometimes necessary to compensate for by the use of large panels of antibodies in order to achieve a confident interpretation in the case of diagnostically problematical tumours. This lack of antibody specificity is one of several limitations of IHC (Table 1.2) which have enabled electron microscopy to survive in the diagnostic analysis of difficult tumours where uncertainty arises from conflicting clinical, histological or immunohistochemical findings. Unusual immunoreactivities can be encountered in tumours: cytokeratin positivity, traditionally the hallmark of carcinoma, can be seen in malignant melanoma, sarcoma and even lymphoma, while smooth-muscle actin can be expected, not only in smooth-muscle tumours, but in neoplasms showing pericytic, myoepithelial, myofibroblastic, endothelial cell and epithelial cell differentiation. In these instances, interpretation becomes uncertain and there is an opportunity for electron microscopy to play a role in clarifying the diagnosis.

1.4 Value of Electron Microscopy in Addition to Diagnosis

It is recognised that tumours are diagnosed with a certain level of confidence, and that this can be increased by electron microscopy. This is important primarily because there is nothing so important for the clinician managing a cancer patient as to have a diagnosis held with maximum confidence. However, it is also important for pathologists when they attend multidisciplinary team meetings and need to argue their case against conflicting opinions from other clinicians, such as radiologists and surgeons. The enhanced degree of diagnostic precision that electron microscopy can bring also protects pathologists against litigious patients or their relatives, and it has been used in cases where there has been suspected specimen contamination (Zuppan *et al.*, 1997).

Electron microscopy not only promotes the identification of named tumours to help clinicians manage patients, but gives pathologists a better understanding of the nature or cellular differentiation in tumours. Sometimes this information may not be diagnostically relevant and will appeal to the academically or research-orientated pathologist wishing to engage in publication. It can, however, also be helpful for junior pathologists who can see in electron microscopy images instructive correlations with the more familiar light microscopy images.

It should be noted that electron microscopy, like IHC, is a technique which provides information on differentiation, and not necessarily cell of origin. Many early papers refer to the value of electron microscopy in defining histogenesis (for example, Miettinen *et al.*, 1984), and yet, currently, our thinking is more towards the concept of a stem cell origin for many tumours (Dirks, 2010; Huysentruyt and Seyfried, 2010). Here, a stem cell, which under normal conditions would develop into a keratinocyte or melanocyte in the skin, for example, undergoes mutation and uncontrolled proliferation to produce a cutaneous squamous-cell carcinoma or malignant melanoma. Therefore, while the tumour may have many of the features of the tissue which is the intended (normal), non-mutated fate of a given stem cell, the tumour's cell of origin may be a quite different cell in terms of differentiation.

For those investigators engaged in cancer research, including the development of anti-cancer therapies (Gilloteaux *et al.*, 2001), electron microscopy of the source tissue – the *in vivo* tumour – provides a base-line for assessing the nature and cellular differentiation of their experimental material. Techniques include the use of cell lines or cells grown in culture (Nagashima *et al.*, 1990; Hara *et al.*, 1991; Carlén *et al.*, 1992; Hiraiwa *et al.*, 1997; Yufu *et al.*, 1999), the growth of solid tumour *in vitro* (Conley *et al.*, 1976; Xiang *et al.*, 2011), including the use of artificial matrices (Benton *et al.*, 2011), and the growth of tumour xenografted into experimental animals (Llombart-Bosch *et al.*, 1989; Hayashi and Akagi, 2000; Malham *et al.*, 2001; Hingorani *et al.*, 2011). Electron microscopy in these situations provides an opportunity of confirming the expression of cell features expected from the original *in vivo* tumour, but also of detecting culturing artefacts as part of the neo-differentiation that can occur when tumour cells are placed in a new (*in vitro*) environment. An example includes the bundles of myofilaments (Tsokos *et al.*, 1987), sometimes with fibronexus junctions (Lawton *et al.*, 1994), which form as cells attach to a physical substratum.