



# Chapter 4

## An approach to Dermatopathology: Immunohistochemical and special stains

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When viewing pathology specimens under the microscope, it is important to first orient yourself to the specimen and then:

1. Be able to differentiate whether it was a shave or punch biopsy
2. Decipher the location based on what you see
  - (a) Thick stratum corneum and presence of stratum lucidum: acral

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- (b) Thin skin with loose connective tissue: eyelid
  - (c) Terminal hair follicles: scalp, axilla vs. vellus hair follicles: face
  - (d) Sebaceous glands: face
  - (e) Absent stratum corneum or granulosum: mucosa
3. Visualize all layers present including the epidermis, dermis, subcutaneous fat
  4. Locate areas with main pathology

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### Descriptive Terms

<b>Term</b>	<b>Description</b>
Acantholysis	Separation of epidermal keratinocytes due to destruction of desmosomes
Acanthosis	Hyperplasia of epidermis
Atrophy	Chronic degeneration of the epidermis or dermis resulting in thinning and fragility of structure
Dyskeratosis	Abnormal keratinization of a keratinocyte while the cell is still in the epidermis
Epidermotropism	The propensity of malignant lymphocytes to migrate into the epidermis without significant spongiosis
Exocytosis	The presence of inflammatory cells, such as benign lymphocytes, neutrophils or eosinophils, within the epidermis during an inflammatory reaction
Fibrosis	An increase in both individual collagen fiber thickness and in overall collagen density within the dermis

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Hyperkeratosis	An increase in the thickness of the stratum corneum
Interface changes	Constellation of changes seen at the dermoepidermal junction in interface dermatitis, including formation of vacuoles in the basal keratinocytes, dying individual keratinocytes, and lymphocyte exocytosis
Interstitial	Located in the spaces between the collagen and elastic fibers of the dermis
Lichenoid	Having a band-like infiltrate of inflammatory cells (usually lymphocytes) directly beneath the epidermis and masking the dermoepidermal junction
Papillomatosis	Undulating epidermal projections resembling fingers or church spires
Parakeratosis	Retention of the nucleus within the stratum corneum cells instead of loss of the nucleus
Pigment incontinence	Loss of melanin, which is normally found in epidermal keratinocytes and melanocytes, into the dermis where it is phagocytosed by melanophages
Pseudoepitheliomatous hyperplasia	Marked irregular acanthosis of the epidermis that is so severe as to mimic squamous cell carcinoma
Psoriasisiform	Having epidermal hyperplasia featuring long, thin, regular rete ridges that resemble those seen in classic plaque-type psoriasis

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Solar elastosis	Gray-blue discoloration of superficial dermal elastic fibers secondary to sun damage
Spongiosis	Edema between epidermal keratinocytes, pushing them apart and straining intercellular bridges
Vasculitis	Endothelial cell swelling, fibrinoid necrosis of the vessel wall, and infiltration of the vessel wall by inflammatory cells
Vasculopathy	Any abnormality of the vessel wall that does not meet the criteria of vasculitis

**Melanocytic Markers**

<b>Marker</b>	<b>Pattern of Staining</b>	<b>Cell Types Positive</b>	<b>Common Uses in Dermopath</b>
S-100	Nucleus & cytoplasm	Melanocytes, nerve sheath cells, some histiocytes	Melanocytic nevi and melanoma, neural tumors, Langerhans cells, granular cell tumors, Rosai-Dorfman disease
MART-1 (Melan-A)	Cytoplasm	Melanocytes	Melanocytic nevi and melanoma
SOX-10	Nucleus	Melanocytes, nerve sheath cells	Melanocytic nevi and melanoma, neural tumors
MiTF	Nucleus	Melanocytes, nerve sheath cells	Melanocytic nevi and melanoma
HMB-45	Cytoplasm	Melanocytes	Melanocytic nevi and melanoma

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**Epithelial markers**

<b>Marker</b>	<b>Pattern of staining</b>	<b>Cell types positive</b>	<b>Common uses in Dermopath</b>
High Molecular Weight Cytokeratins	Cytoplasm	Epithelial cells of epidermis, hair follicle, sebaceous gland, eccrine and apocrine ducts	BCC and SCC (+), adnexal tumors variable
Low Molecular Weight Cytokeratins	Cytoplasm	Epithelial cells of epidermis, hair follicle, sebaceous gland, eccrine and apocrine glands	Adnexal tumors (+), metastatic carcinoma usually (+)
CAM5.2	Cytoplasm	Epithelial cells of epidermis, hair follicle, sebaceous gland, eccrine and apocrine glands	Eccrine and apocrine secretory coils (+), Paget's disease (+), SCC (-)
Epithelial Membrane Antigen (EMA)	Cytoplasm	Epithelial cells and ducts of sebaceous gland, eccrine and apocrine glands	(+) in SCC, sebaceous tumors, and eccrine and apocrine adnexal tumors, (-) in BCC
Carcinoembryonic Antigen (CEA)	Ductal/luminal cytoplasm	Ductal/luminal surfaces of eccrine and apocrine ducts and glands	Identifying ductal differentiation in tumors such as microcystic adnexal carcinoma (+)

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BerEP4	Cell membrane	Epithelial cells of some vellus hair follicles, eccrine and apocrine coils	Distinguishing BCC (+) from SCC (-) and microcystic adnexal carcinoma (-)
P63	Nucleus	Epithelial cells of epidermis, hair follicle, myoepithelial cells of eccrine and apocrine glands and ducts	SCC (+), distinguishing primary skin adnexal tumors (+) from metastatic adenocarcinoma (-)

**Mesenchymal markers**

<b>Marker</b>	<b>Pattern of staining</b>	<b>Cell types positive</b>	<b>Common uses in Dermath</b>
Factor XIIIa	Cytoplasm	Dermal dendritic cells, fibroblasts	Dermatofibroma (+) vs. dermatofibrosarcoma protuberans (-)
CD34	Cytoplasm	Endothelial cells, dermal dendritic cells	Vascular tumors, dermatofibrosarcoma protuberans, spindle cell lipoma
CD31	Cytoplasm	Endothelial cells	Vascular tumors
D2-40	Cytoplasm	Endothelial cells of lymphatics	Lymphatic tumors
SMA	Cytoplasm	Smooth muscle, myofibroblasts	Smooth muscle tumors, nodular fasciitis, glomus tumor

Desmin	Cytoplasm	Smooth and skeletal muscle	Tumors of smooth and skeletal muscle
Vimentin	Cytoplasm	All cell types of mesenchymal derivation	Usually carcinoma (-), sarcoma (+)
Neuron-specific Enolase	Cytoplasm	Nerves and neuroendocrine cells	Identifying neural and neuroendocrine tumors such as granular cell tumor (+)

#### Hematopoietic markers

Marker	Pattern of staining	Cell types positive	Common uses in Dermath
CD45/LCA	Membrane/cytoplasm	Lymphocytes, neutrophils, eosinophils, histiocytes, plasma cells	Establish the hematopoietic origin of a tumor/infiltrate
CD3	Membrane/cytoplasm	T-lymphocytes	Identifying T-lymphocytes
CD20	Membrane/cytoplasm	B-lymphocytes	Identifying B-lymphocytes
CD4	Membrane/cytoplasm	Helper T-lymphocytes	Identifying helper T-lymphocytes
CD5	Membrane/cytoplasm	T-lymphocytes	T cell marker, loss of CD5 can indicate possible malignancy

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CD7	Membrane/ cytoplasm	T-lymphocytes	T cell marker, loss of CD7 can indicate possible malignancy
CD8	Membrane/ cytoplasm	Cytotoxic T-lymphocytes	Identifying cytotoxic T-lymphocytes
CD30	Membrane/ cytoplasm	Activated T-lymphocytes and neoplastic T-lymphocytes	Increased expression in lymphomatoid papulosis and anaplastic large T-cell lymphoma
CD68	Membrane/ cytoplasm	Histiocytes/ macrophages	Identifying histiocytes
CD38/138	Membrane/ cytoplasm	Plasma cells	Identifying plasma cells
CD56	Membrane/ cytoplasm	NK/T cells, neuroendocrine cells	Identifying NK-cell differentiation, also in neuroendocrine tumors
BCL-2	Cytoplasm	T-lymphocytes, B-lymphocytes outside of germinal centers	Marginal zone lymphoma, diffuse large B-cell lymphoma, absent in most cases of primary cutaneous follicle center lymphoma vs. strong positive reaction in nodal follicular lymphoma

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BCL-6	Nucleus	B-lymphocytes within germinal centers, some T-lymphocytes	Follicle center lymphoma
CD10	Membrane/ cytoplasm	Germinal center B-cells, sebaceous glands	Follicle center lymphoma (+), also (+) in atypical fibroxanthoma and clear cell renal cell carcinoma
MUM-1	Nucleus	Plasma cells and post-germinal center B-lymphocytes	Diffuse large B-cell lymphoma, myeloma
Kappa light chain	Cytoplasm	Plasma cells	Marginal zone lymphoma
Lambda light chain	Cytoplasm	Plasma cells	Marginal zone lymphoma
ALK-1	Nucleus and cytoplasm	Anaplastic large T-cell lymphoma (ALCL) cells	Usually systemic ALCL (+) vs. primary cutaneous ALCL (-)

#### Miscellaneous IHC markers

Marker	Pattern of staining	Cell types positive	Common uses in Dermath
CK7	Cytoplasm	Tissue of breast, lung, upper GI tract and bladder	Identifying adenocarcinomas of breast, lung, upper GI tract and bladder, (+) in Toker cells, mammary and extramammary Paget's disease

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CK20	Cytoplasm	Merkel cells, adenocarcinomas of colon, bladder and bile ducts	Merkel cell carcinoma, identifying adenocarcinomas of colon, some cases of extramammary Paget's disease secondary to underlying carcinomas
CD1a	Membrane/cytoplasm	Langerhans cells	Identifying Langerhans cells
CD117 (c-kit)	Membrane/cytoplasm	Mast cells	Identifying mast cells
TTF-1	Nuclear	Lung carcinoma, thyroid carcinoma	Thyroid cancer, distinguishing between small cell carcinoma of the lung and Merkel cell carcinoma
Adipophilin	Perivacuolar	Sebaceous gland cells	Establishing sebaceous differentiation in a tumor, sebaceous tumors
GCDFP-15	Cytoplasm	Breast ducts	Identifying breast carcinoma, mammary and extramammary Paget's disease
Chromogranin	Cytoplasm	Neuroendocrine cells and tumors	Identifying neuroendocrine tumors such as Merkel cell carcinoma

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Synaptophysin	Cytoplasm	Neuroendocrine cells and tumors	Identifying neuroendocrine tumors such as Merkel cell carcinoma
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### Special stains

Marker	Material staining	Color of positive stain	Common uses in Dermatopath
PAS	Fungal structures, basement membrane, glycogen, epithelial mucins	Dark pink/red	Dermatophytes and deep fungal infections
GMS	Fungal structures	Black	Fungal infections
Alcian Blue	Stromal/mesenchymal mucin	Bright blue	Dermal mucin deposition
Colloidal Iron	Mucins, some stromal and some epithelial	Blue	Dermal mucin deposition
Masson Trichrome	Stain that highlights collagen and muscle	Collagen: blue/green, muscle fibers/keratin: red	Identifying collagen and fibrosis
Fontana Masson	Melanin	Black granules	Distinguishing between hemosiderin (-) and melanin pigment (+)
Perl's Prussian Blue	Iron/hemosiderin	Blue granules	Distinguishing between hemosiderin (+) and melanin pigment (-)

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Chloroacetate esterase (Leder stain)	Mast cell granules	Purple	Identifying mast cells
Gram	Bacteria	Gram+ organisms: dark blue/purple, Gram-organisms: red	Identifying bacteria
Fite	Mycobacteria	Red	Identifying mycobacteria
Warthin Starry	Spirochetes, some bacteria	Black	Syphilis, bacillary angiomatosis
Toluidine Blue	Mast cells, mucin	Mast cells: purple, mucin: purple/red	Identifying mast cells
Congo Red	Amyloid	Red/orange	Identifying amyloid
Crystal Violet	Amyloid	Violet/purple	Identifying amyloid
Verhoeff-Van Gieson	Elastin	Black	Identifying elastic fibers
Von Kossa	Calcium	Black	Identifying calcium, calciphylaxis, pseudoxanthoma elasticum
Oil Red O	Lipid (fresh or frozen tissue only)	Red	Identifying lipids and fat
Sudan Black B	Lipid	Black	Identifying lipids and fat
Giemsa	Mast cells	Dark blue/purple	Identifying mast cells

## Immunofluorescence Studies

Direct immunofluorescence (DIF): An antibody directly detects presence of a pathologic antibody in the skin

Indirect immunofluorescence (IIF): Serum is introduced to a substrate in order to detect circulating antibodies

Salt-split skin test: with NaCl, skin is cleaved at lamina lucida at BMZ – Allows separation of roof/floor fluorescence

Biopsy sites for DIF: autoimmune blistering diseases – inflamed but unblistered perilesional skin, autoimmune and inflammatory diseases other than blistering diseases – lesional skin, vasculitis- 1 to 2 day-old fresh lesional skin

<b>Disease</b>	<b>Pattern of DIF Staining</b>	<b>Salt Split IF</b>
Pemphigus vulgaris	IgG and occasional C3 in the intercellular region of the epidermis	
Pemphigus foliaceus	IgG and occasional C3 in the intercellular region of the epidermis	
Pemphigus erythematosus	Intercellular and basement membrane staining with IgG and/or C3	
Paraneoplastic pemphigus	Intercellular and basement membrane staining with IgG and/or C3	
IgA pemphigus	Intercellular deposition of IgA in the epidermis	
Bullous pemphigoid	Linear, homogeneous deposition of IgG and/or C3 at the BMZ	Roof
Epidermolysis bullosa acquisita	Linear deposition of IgG (less commonly C3, IgA or IgM) at the BMZ	Floor

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<b>Disease</b>	<b>Pattern of DIF Staining</b>	<b>Salt Split IF</b>
Bullous SLE	IgG (less commonly IgA, IgM) and complement at the BMZ	Floor
Mucous membrane pemphigoid	Linear deposit of IgG (and sometimes IgA) and C3 at the BMZ	Roof/ Floor
Linear IgA bullous dermatosis	Homogeneous linear pattern of IgA deposition at the BMZ	Roof/ Floor
Dermatitis herpetiformis	Granular deposits of IgA in the dermal papillae	
Porphyria cutanea tarda	Ig, complement and fibrinogen at the BMZ and around blood vessels	
Henoch-Schönlein purpura	IgA (usually fibrinogen and C3 as well) deposition in blood vessels	
Lichen planus	Shaggy deposits of fibrin at the BMZ	

## References

1. Molina-Ruiz AM, Fuertes L, Requena L. Immunohistology and molecular studies of sweat gland tumors. In: Plaza JA, Prieto VG, editors. Applied immunohistochemistry in the evaluation of skin neoplasms. Switzerland: Springer International Publishing Switzerland; 2016.
2. Sanders DSA, Carr RA. The use of immunohistochemistry in the differential diagnosis of common epithelial tumors of the skin. *Curr Diagn Pathol.* 2007;13:237–51.
3. Compton LA, Murphy GF, Lian CG. Diagnostic immunohistochemistry in cutaneous neoplasia: an update. *Dermatopathology.* 2015;2:15–42.