CYTOKERATINS

Characteristics

Cytokeratins (CKs; also named keratins) are a large family of intermediate sized (~10 nm in diameter) cytoskeletal filaments, essential for normal structure and function of all mammalian epithelial cells. The CK filaments are obligatory heterodimers each formed tetrameric by two different proteins, one acidic (type I) and one basic-to-neutral (type II) CK protein. Currently at least 54 human CK types have been identified. The CKs are numbered according to the nomenclature proposed by a consensus group in 2006 based on the 1982 catalogue of Moll (1-4). The new consensus nomenclature recognizes 28 type I CKs (CK9–CK10, CK12–CK20, CK23–CK28, CK31–CK40) and 26 type II CKs (CK1–CK8, CK71–CK86). Of these, 37 are epithelial ('soft CKs') while the rest belong to hair and nail ('hard CKs'). High molecular weight (HMW) CKs vary from 48 to 66 kDa, low molecular weight (LMW) CKs from 40 to 46 kDa. The smaller and acidic type 1 keratins (CK9–CK20) are encoded on chromosome 17q, while the larger and more basic type II keratins (CK1–CK8) are encoded on chromosome 12q. The basic member of each keratin pair is always larger than the acidic member, on average approximately 8 kDa. From two to ten CK types are found in highly specific patterns in the individual cells related to their type and stage of differentiation. The CK types most relevant for immunohistochemistry are listed in Table 1 and described below.

Table 1. The diagnostically most important CK types and their distribution in normal cells.
CKs 4, 13, 5 and 14 are HMW CKs, while CKs 17, 7, 20, 8, 18 and 19 are LMW CKs.

Type I CK		**CK13**		**CK14**	**CK17**		**CK20**	**CK18**	**CK19**
Type II CK	**CK4**		**CK5**			**CK7**		**CK8**	
Squamous epithelium, nonkeratinizing, suprabasal cells	+++	+++	++	++	(+)[1]	-	-	-	-
Squamous epithelium, basal cells	-	-	+++	+++	(+)[1]	(+)[2]	-	(+)[2]	++
Urothelium, basal cells	+	+++	+++	-	++	+++	-	+++	+++
Urothelium, intermediate cells	-	++	++	-	+	+++	+	+++	+++
Urothelium, superficial cells	-	-	-	-	-	+++	++	+++	+++
Placental trophoblasts	-	+?	+++	+?	+?	+++	-	+++	+++
Mesothelium	-	-	++	++	+	+++	-	+++	+++
Complex epithelia*, basal/myoepithelial cells	-	-	+++	+++	+++	-	-	++	++
Complex epithelia*, luminal cells	-	-	+	+	+	(+++)[3]	-	+++	+++
Simple epithelia of lung alveoli, biliary tract, pancreatic ducts, endometrium, Fallopian tube, renal collecting ducts	-	-	(+)[4]	-	(+)[4]	++	-	+++	+++
Simple epithelia of stomach and intestine	-	-	-	-	-	(+)[5]	++	+++	+++
Simple neuroendocrine epithelia	-	-	-	-	-	(+)[6]	(+)[7]	++	(++)[8]
Simple epithelia of hepatocytes, pancreatic acini, proximal renal tubules, adrenal cortex	-	-	-	-	-	-	-	++	-
Smooth muscle cells, myofibroblasts, dendritic reticular cells, endothelia	-	-	-	-	-	(+)[9]	-	+[10]	+

*Pseudostratified in respiratory tract, two-layered in glandular ducts. ? Conflicting evidence. +++: large amounts in most cells, ++: moderate or varying amounts, +: scares amounts, (): occurrence primarily in certain cells/organs, see footnotes: 1. Pilosebaceous tract, foetal and regenerating epidermis; 2. Lymphocyte rich epithelia, e.g. tonsil; 3. Prostate CK7 neg.; 4. Pancreas; 5. Gastric foveolae; 6. Thyroid gland; 7. Merkel cells, corticotropic adenopituitary cells, scattered endocrine cells in the gastrointestinal tract; 8. Adenopituitary cells and scattered endocrine cells in gastrointestinal tract, 9. Venolar endothelium, 10. Smooth muscle cells, most pronounced in myometrium.

Cytokeratins in normal tissues (Table 1)

LMW CKs include the type I CK17, K20, CK18 and CK19, and the type II CK7 and CK8. CK8 and CK18 are almost always paired, designated CK8/18. They are the first to appear in embryogenesis and the most widespread CKs in the differentiated cells, representing primary (constitutive) CKs of simple epithelia. As outlined in Table 1, CK8/18 are the only CKs expressed in some simple epithelia (hepatocytes, pancreatic acini, proximal renal tubules, adrenal cortex), while in most others also the secondary CKs CK19 and either CK20 (terminal differentiation in gastrointestinal cells) or CK7 are found. CK8/18, CK19 and CK7 are also found in complex (pseudostratified) epithelia (mainly the luminal compartment apart from prostate), urothelium (together with CK20 in cells of terminal differentiation), non-keratinizing squamous epithelium (focally), mesothelium and few mesenchymal cell types. CK17 has a special expression pattern, occurring particularly in basal cells of complex epithelia, in urothelium, and in pilosebaceous cells.

HMW CKs include the type I CK13 and CK14, and the type II CK4 and CK5 (Table 1). CK5/14 are typically paired, representing the primary (constitutive) CKs of basal cells in squamous and complex epithelia. At divergence, in urothelium CK5 is paired with CK13. When maturing, the nonkeratinizing squamous epithelium expresses CK4 and CK13, while the urothelium only expresses LMW CKs, and the keratinizing squamous epithelium express CK1 and CK10 (which are not included in Table 1 as they have little diagnostic relevance).

Table 2. The diagnostically most important CK types and their distribution in malignant epithelial neoplasms (carcinomas, epithelioid malignant mesothelioma and endocrine neoplasms).

Type I CK		**CK13**		**CK14**	**CK17**		**CK20**	**CK18**	**CK19**
Type II CK	**CK4**		**CK5**			**CK7**		**CK8**	
Squamous cell carcinoma	+/-	+/-	+	+/-	+/-	-/+	-	-/+	+/-
Urothelial carcinoma	+/-	+/-	+/-	+/-	+/-	+	-/+	+	+
Epithelioid malignant mesothelioma	-	-	+/-	+	+/-?	+/-	-	+	+
Lung adenocarcinoma	-	-	-	-	-/+	+/-	-(+)[1]	+	+
Breast and salivary gland adenocarcinoma	-	-	-/+[2]	-/+[2]	-(+)[2]	+	-	+	+
Oesophagus, stomach, biliary tract, pancreas, endometrioid and ovarian non-mucinous adenocarcinoma	-	-	-(+)[3]	-	-(+)[3]	+/-	-/+[4]	+	+
Small intestine, appendix, colon, rectum and ovarian mucinous adenocarcinoma	-	-	-	-	-	-(+)	+/-	+	+
Renal cell and prostate adenocarcinoma	-	-	-	-	-	-(+)[5]	-	+	+/-
Neuroendocrine neoplasms	-	-	-	-	-	-/+	-(+)[6]	+	+
Thyroid carcinoma	-	-	-	-	-	+	-	+	+/-
Hepatocellular carcinoma	-	-	-	-	-	-(+)	-	+	-(+)
Adrenocortical carcinoma and germ cell tumours	-	-	-	-	-	-	-	+	-

Occurrence (positivity >10% of cells) in tumours: +: >90% , +/-: 50-90%, -/+: 10-50%, -(+): 1-10%, -: <1%. 1. Intestinal differentiation; 2. Mainly metaplastic/basal like, 3. Mainly pancreas and ovarian serous carcinoma. 4. Usually focal positivity. 5. Papillary and chromophobic renal cell carcinoma mostly positive, prostate adenocarcinoma positive in about 10%. 6. Merkel cell carcinoma mostly positive.

Cytokeratins in neoplastic lesions (Table 2)

Primary and metastatic carcinomas, mesotheliomas and endocrine neoplasias tend to retain the CK profiles of the putative cell of origin (Table 2). Thus, the large majority of squamous cell and urothelial carcinomas as well as mesotheliomas express CK5, the large majority colorectal adenocarcinomas are CK20[+]/CK7[-], and the large majority of hepatocellular, renal cell, prostate and adrenal cortical carcinomas solely express CK8/18 (2,5,7). However, carcinomas may lose 'maturation types' of CKs: Squamous cell carcinomas tend to lose CK4/13 (that are expressed in normal suprabasal squamous epithelium). Urothelial carcinomas typically express both HMW and LMW CKs but often lose one or more types (e.g., CK5, CK7 and/or CK20). At the same time squamous cell carcinomas tend to express LMW CKs (that are only focally expressed in normal squamous epithelium). Colorectal adenocarcinomas may lose CK20 and may gain CK7 (but the individual tumour rarely do both). Thyroid carcinoma often express CK19 (which is not present in the normal gland).

Application

Immunohistochemical mapping of CK types is important in classification of carcinomas of unknown primary origin.
Identification of LMW CK types such as CK8/18 is also important in the study of neoplastic development and differentiation. E.g., in contrast to normal squamous epithelia, CK8/18 may be widely expressed in squamous cell carcinoma of the uterine cervix and head and neck as well as their precursor lesions (8-10). In intraductal breast lesions and invasive ductal carcinomas, identification of the luminal cell CKs CK8/18, CK7 and CK19, and the basal/myoepithelial cell markers CK5/14 are relevant for subclassification and prognostication (11,12).
Neoplasms derived from simple epithelia, expressing CK8/18 as the only CK types, will often show a low level of CK expression. IHC detection of CK8/18 therefore calls for sensitive and robust immunoassays based on appropriate choice of antibodies (Abs) and protocols calibrated for the purpose.
For most CK types a large number of commercially available Abs are available, and staining protocols can be set up in countless ways making it difficult for laboratories to identify and implement the best immunoassays. Less successful CK Abs and suboptimal protocols may result in ambiguous or false negative staining reactions hampering the diagnostic utility and consequently the reliability of IHC.
In NordiQC, tests for CK5, CK7, CK8/18, CK14, CK19 and CK20 as well as CK-LMW, CK-HMW and CK-PAN have been performed one or more times. For all of them, results regarding good and less successful antibodies are described together with recommendable protocol settings.

Visualisation
See assessments of CK-pan, CK-HMW and CK-LMW.

Controls
On-slide control TMAs for CKs should include normal tissues as listed below.

CK8/18 and CK-LMW: Liver (liver cells are CK low-expressors)
CK19: Colon/appendix (all enterocytes positive) and oesophagus (basal cells positive)
CK20: Colon/appendix (all enterocytes apart from basal crypt cells positive)
CK5/14 and CK-HMW: Pancreas (scattered columnar epithelial cells of intercalated ducts show a weak to moderate predominantly membranous staining reaction).
CK7: Pancreas (the vast majority of epithelial cells of the intercalating ducts shows an at least weak to moderate cytoplasmic staining reaction, whereas the epithelial cells of large pancreatic ducts shows an intense staining reaction, while no staining is seen in the acinar cells).
CK-PAN: Liver (like CK8/18) and pancreas (like CK5/14).

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