Published in final edited form as:
Oncogene. 2011 January 13; 30(2): 127–138. doi:10.1038/onc.2010.456.

Keratins in health and cancer: more than mere epithelial cell markers

V Karantza[1,2]

[1]Department of Medicine, University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School, Piscataway, NJ, USA

[2]Division of Medical Oncology, The Cancer Institute of New Jersey, New Brunswick, NJ, USA

Abstract

Keratins are the intermediate filament (IF)-forming proteins of epithelial cells. Since their initial characterization almost 30 years ago, the total number of mammalian keratins has increased to 54, including 28 type I and 26 type II keratins. Keratins are obligate heteropolymers and, similarly to other IFs, they contain a dimeric central α-helical rod domain that is flanked by non-helical head and tail domains. The 10-nm keratin filaments participate in the formation of a proteinaceous structural framework within the cellular cytoplasm and, as such, serve an important role in epithelial cell protection from mechanical and non-mechanical stressors, a property extensively substantiated by the discovery of human keratin mutations predisposing to tissue-specific injury and by studies in keratin knockout and transgenic mice. More recently, keratins have also been recognized as regulators of other cellular properties and functions, including apico-basal polarization, motility, cell size, protein synthesis and membrane traffic and signaling. In cancer, keratins are extensively used as diagnostic tumor markers, as epithelial malignancies largely maintain the specific keratin patterns associated with their respective cells of origin, and, in many occasions, full-length or cleaved keratin expression (or lack there of) in tumors and/or peripheral blood carries prognostic significance for cancer patients. Quite intriguingly, several studies have provided evidence for active keratin involvement in cancer cell invasion and metastasis, as well as in treatment responsiveness, and have set the foundation for further exploration of the role of keratins as multifunctional regulators of epithelial tumorigenesis.

Keywords

keratins; cancer; invasion; diagnosis; prognosis; drug resistance

Introduction

The cytoskeleton is a proteinaceous structural framework within the cellular cytoplasm (Fuchs and Cleveland, 1998). Eukaryotic cells contain three main kinds of cytoskeletal filaments, namely microfilaments, intermediate filaments (IFs) and microtubules. Microfilaments are composed of 6-nm intertwined actin chains and are responsible for resisting tension and maintaining cellular shape, forming cytoplasmic protuberances and participating in cell–cell and cell–matrix interactions (Pollard and Cooper, 2009). The 10 nm

Correspondence: Dr V Karantza, The Cancer Institute of New Jersey, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, Room 3550, 195 Little Albany Street, New Brunswick, NJ 08903, USA. karantva@umdnj.edu.

Conflict of interest
The author declares no conflict of interest

in diameter IFs are more stable than actin filaments, organize the internal three-dimensional cellular structure and, similarly to microfilaments, also function in cell shape maintenance by bearing tension (Herrmann *et al.*, 2009). Finally, the microtubules are 23 nm in diameter hollow cylinders, most commonly comprising of 13 protofilaments, which in turn are polymers of alpha- and beta-tubulin, and play key roles in intracellular transport and the formation of the mitotic spindle (Glotzer, 2009).

In contrast to actin filaments and microtubules, IFs are encoded by a large family of genes expressed in a tissue- and differentiation state-specific manner and are classified into discrete categories based on their rod domain amino-acid sequence. Types 1 and 2 IFs are found primarily in epithelial cells and include the acidic and basic keratins, respectively; type 3 IFs include vimentin, desmin and glial fibrillary acidic protein; type 4 IFs assemble into neurofilaments; and type 5 IFs are the nuclear lamins (Herrmann *et al.*, 2007). All IFs have a dimeric central rod domain, which is a coiled coil of two parallel α-helices flanked by head and tail domains of variable lengths. Antiparallel molecular dimers (referred to as tetramers) polymerize in a staggered manner to make apolar protofilaments (3 nm in diameter), which in turn associate to protofibrils (4–5 nm in diameter), and then to the 10-nm IFs (Steinert *et al.*, 1993), which are among the most chemically stable cellular structures, resisting high temperature, high salt and detergent solubilization.

In this review, we will focus on keratins, the IFs of epithelial cells and will review their functional role in the normal epithelium and their emergent significance in the pathophysiology and treatment of epithelial malignancies.

Keratins in health

Epithelial cell IFs

Keratins are the IF-forming proteins of epithelial cells and account for the majority of *IF* genes in the human genome. Two-dimensional isoelectric focusing and sodium dodecyl sulfate–polyacrylamide gel electrophoresis were initially used to profile the keratins of normal human epithelial tissues, cell cultures and tumors (Moll *et al.*, 1982), and resulted in the first comprehensive keratin catalog, which included 19 members and separated them into type I (or acidic, K9–K19) and type II (or basic to neutral, K1–8) keratins, and the recognition that keratin filaments form by heterotypic pairing of type I and type II proteins at equimolar amounts (Quinlan *et al.*, 1984). Additional keratins were subsequently identified (Moll *et al.*, 1990; Takahashi *et al.*, 1995), including a large number of hair follicle-specific keratins (Winter *et al.*, 1998). Given the completion of the human genome sequence, a consensus nomenclature for mammalian keratin genes and proteins is now in place (Schweizer *et al.*, 2006) and includes 28 type I (20 epithelial and 11 hair) keratins and 26 type II (20 epithelial and six hair) keratins. Similarly to other IFs, keratins contain a central α-helical domain of about 310 amino acids, which is composed of subdomains 1A, 1B, 2A and 2B connected by linkers L1, L12 and L2 (Figure 1). The non-helical head and tail domains consist of subdomains V1 and H1 and H2 and V2, respectively (Herrmann *et al.*, 2007).

Keratins are expressed in all types of epithelial cells (simple, stratified, keratinized and cornified) (Moll *et al.*, 1982; Bragulla and Homberger, 2009). K8 and its obligate partner K18 constitute the primary keratin pair in many simple epithelial cells, such as hepatocytes, pancreatic acinar and islet cells, and proximal tubular kidney epithelial cells, and are also found in pseudostratified (for example, respiratory) and complex (for example, glandular) epithelia (Figure 2) and the urothelium; K7 and K19 are expressed in other simple epithelial cells, such as duct-lining cells, intestinal cells and mesothelial cells, in pseudostratified epithelia and the urothelium; K20 is expressed in gastrointestinal epithelia, the urothelium

and in Merkel (neuroendocrine) cells of the skin; K5 and K14 form the main keratin pair in keratinocytes of stratified squamous epithelia, and are also expressed in basal and myoepithelial cells of complex and glandular epithelial tissues; K6 and K16 have been identified in the epidermis, nail epithelia and non-keratinizing stratified squamous epithelia, but are also expressed in ductal luminal cells and in secretory cells of human eccrine sweat glands; K17 is a basal/myoepithelial cell keratin that is characteristically induced after skin injury; K1/K10, K15, K9 and K2 are all expressed in keratinocytes; K3 and K12 are the keratins of corneal epithelium; K4 and K13 are characteristic of mucosal stratified squamous epithelial cells; and K25–K28 and K71–K75 are hair-follicle-specific keratins, whereas K31–K40 and K81–K86 are keratins of the hair fiber (Moll *et al.*, 2008).

Protectors of epithelial cell integrity and much more, and so on

Keratins serve an important role in epithelial cell protection from mechanical and non-mechanical stressors (Coulombe and Omary, 2002), a function that has been particularly well characterized in the skin, cornea and liver by both the discovery of human keratin mutations predisposing to tissue-specific injury (Omary *et al.*, 2009) and the development of keratin knockout (KO) and transgenic mouse models (Vijayaraj *et al.*, 2007). For example, mutations in keratins K5 or K14, which are expressed in the basal cells of stratified epithelial, lead to physical trauma-induced fragility and lysis of these cells resulting in intraepithelial blisters and epidermolysis bullosa simplex (Bonifas *et al.*, 1991; Coulombe *et al.*, 1991; Lane *et al.*, 1992); mutations in the cornea-specific keratins K3 or K12 result in the fragility of the anterior corneal epithelium and intraepithelial microcyst formation (Meesmann's corneal dystrophy) (Irvine *et al.*, 1997); K8 (Ku *et al.*, 2001) or K18 (Ku *et al.*, 1997) mutations are found as a predisposition to chronic (Omary *et al.*, 2009) and acute (Strnad *et al.*, 2010) liver disease. Similarly, K5-null mice survive for only a few hours after birth and exhibit extensive skin blistering (Peters *et al.*, 2001), whereas K14-null mice show a similar, but less severe, phenotype and die within 3–4 days (Lloyd *et al.*, 1995); K12 KO mice show corneal erosions (Kao *et al.*, 1996); K8 deficiency results in liver hemorrhage and embryonic lethality in C57BL/6 mice (Baribault *et al.*, 1993) together with mechanical fragility and susceptibility to hepatocyte injury during liver perfusion (Loranger *et al.*, 1997), but causes colorectal hyperplasia and inflammation, rectal prolapse and mild liver injury in the FVB strain (Baribault *et al.*, 1994; Habtezion *et al.*, 2005), whereas K18 KO mice exhibit late-onset, subclinical liver pathology (Magin *et al.*, 1998), suggesting different K8 and K18 roles in inflammatory bowel and liver diseases, although K8 mutations do not appear to be associated with human inflammatory bowel disease (Buning *et al.*, 2004; Ku *et al.*, 2007). Transgenic mouse models have yielded complementary results, further underscoring the functional significance of keratins in epithelial health preservation. Mice overexpressing human K18 bearing an Arg89-to-Cys mutation (corresponding to a highly conserved and mutation 'hot-spot' arginine residue in human skin disorders) show liver and pancreatic keratin filament disruption, hepatocyte fragility, chronic hepatitis (Ku *et al.*, 1995) and increased susceptibility to a variety of stresses, including hepatotoxic drugs, partial hepatectomy, collagenase liver perfusion and Fas-mediated apoptosis (Ku *et al.*, 1999, 2003b; Zatloukal *et al.*, 2000). Overexpression of the human liver disease-associated K8 Gly61-to-Cys variant results in stress-induced liver injury and apoptosis, and a similar phenotype is observed in transgenic mice overexpressing mutant K8 Ser73-to-Ala (Ku and Omary, 2006).

In addition to their widely accepted role as protectors of epithelial cell integrity under a variety of stressful conditions, keratins have been more recently recognized as important regulators of diverse cellular functions, such as apico-basal polarization (Oriolo *et al.*, 2007), cell size determination and protein translation control (Kim *et al.*, 2006; Vijayaraj *et*

al., 2009), organelle positioning and membrane protein targeting (Styers *et al.*, 2005; Toivola *et al.*, 2005).

Post-translational modifications and protein interactions

In response to stress, keratin expression is commonly altered (Toivola *et al.*, 2010) and keratins become post-translationally modified and structurally reorganized (Ku and Omary, 2006). In keratinocytes, stretching results in K10 suppression and K6 induction (Yano *et al.*, 2004), and wounding upregulates K6, K16 and K17 (Kim *et al.*, 2006). In the liver, K8/K18 levels increase about threefold (mRNA and protein) in response to injury, as noted in mice treated with agents that induce Mallory–Denk body formation (Zatloukal *et al.*, 2007) and in the hepatoma cell line HepG2 treated with doxorubicin (Wang *et al.*, 2009; Hammer *et al.*, 2010), and two- to fourfold (protein) in patients with primary biliary cirrhosis (Fickert *et al.*, 2003). In alveolar epithelial cells, shear stress causes structural remodeling of the keratin IF network (Felder *et al.*, 2008; Sivaramakrishnan *et al.*, 2008), whereas hypoxia results in network disassembly and K8/K18 degradation (Na *et al.*, 2010). Similarly, the keratin cytoskeleton disintegrates in mammary epithelial cells under metabolic (combined glucose and oxygen deprivation mimicking the tumor microenvironment; Nelson *et al.*, 2004) stress (Kongara *et al.*, 2010). For mechanical stress, and likely for other types of stress, keratin reorganization involves a temporal sequence of changes that is dependent on stress duration, and consequently stress severity. For example, in alveolar epithelial cells, thin keratin filaments coalesce to tonofibrils/keratin bundles after 1–4 h of shear stress; Mallory-like body formation is observed after 12–16 h of shear stress, and finally, the keratin network collapses by disassembly and ubiquitin-mediated degradation after 24–36 h of shear stress (Ridge *et al.*, 2005; Sivaramakrishnan *et al.*, 2009).

Keratin reorganization under stress is regulated by post-translational modifications and differential keratin association with scaffolding proteins (Coulombe and Omary, 2002). Among the different types of protein modification, phosphorylation is considered a major regulator, as it modulates intrinsic keratin properties, such as solubility, conformation and filament structure, and it also regulates other post-translational modifications (Omary *et al.*, 2006). Keratin phosphorylation occurs at Ser > Thr > Tyr residues within Arg-rich Ser-Arg-**Ser**-Xaa (Xaa denotes any amino acid) or Leu-Leu-**Ser/Thr**-Pro-Leu motifs, among others, located within the head and tail keratin domains. The number of phosphates per keratin molecule is keratin-, cell type- and biological context dependent. In general, phosphorylation levels are low in basal conditions, but increase several fold in mitosis (Toivola *et al.*, 2002) and under a variety of cellular stresses, including drug-induced apoptosis (Liao *et al.*, 1997; Schutte *et al.*, 2004), heat stress (Liao *et al.*, 1997), treatment with phosphatase inhibitors (Toivola *et al.*, 2002), shear stress (Ridge *et al.*, 2005) and metabolic stress (Kongara *et al.*, 2010). Three human K8 (S23, S73, S431) and two K18 (S33, S52) major *in vivo* phosphorylation sites have been characterized (Ku and Omary, 2006), and potentially involved kinases have been primarily determined by *in vitro* studies and appear to be at least partially stress dependent. For example, K8 phosphorylation at Ser73 is mediated by the two stress-activated mitogen-activated protein kinase family members p38 and c-jun-N-terminal kinase in response to the phosphatase inhibitors okadaic acid and orthovanadate (Ku *et al.*, 2002a; Woll *et al.*, 2007) and upon stimulation of the proapoptotic receptor Fas/CD95/Apo-1 (He *et al.*, 2002), respectively, and by protein kinase Cδ (PKCδ) under mechanical stress (Ridge *et al.*, 2005). Phosphorylation regulates the distribution of keratins into an ‘insoluble’ filamentous cytoskeletal pool and a ‘soluble’ cytosolic or detergent-extractable hyperphosphorylated pool (Omary *et al.*, 1998) and plays a role in keratin ubiquitination and turnover by the proteasome (Ku and Omary, 2000; Jaitovich *et al.*, 2008), and likely by autophagy (Kongara *et al.*, 2010). Phosphorylation is also important for the interaction of keratins with keratin-associated proteins, among them

the adapter/signaling 14-3-3 proteins, which are involved in keratin solubilization during mitosis by binding to phospho(Ser33)-K18 and undergoing nuclear-to-cytoplasmic redistribution (Ku *et al.*, 2002b) and in serum-dependent Akt/mTOR (mammalian target of rapamycin) pathway activation by binding to phospho (Ser44)-K17 and again relocating from the nucleus to the cytoplasm (Kim *et al.*, 2006).

Keratins in cancer

Diagnostic markers in epithelial tumors

Given the characteristic cell type-, differentiation- and functional status-dependent keratin expression patterns in epithelial cells, the availability of specific keratin antibodies, and the fact that epithelial tumors largely maintain the features of specific keratin expression associated with the respective cell type of origin, keratins have long and extensively been used as immunohistochemical markers in diagnostic tumor pathology (Figure 3; Table 1) (Moll *et al.*, 2008).

Adenocarcinomas, that is, epithelial cancers arising in glandular tissues, comprise the largest group of human epithelial malignancies and may arise in different organs. The ability to differentiate adenocarcinomas according to their tissue of origin is essential for the selection of the most appropriate treatment regimens, and simple epithelial keratins are the markers predominantly used for this purpose. Most adenocarcinomas express the simple epithelial keratins K8, K18 and K19, whereas K7 and K20 expression is variable. Keratin typing is of particular diagnostic significance in the case of colorectal adenocarcinomas, which similarly to the normal gastrointestinal epithelium are almost always K20-positive, but K7-negative (or have lower K7 expression compared with K20) (Moll *et al.*, 2008). K20 and K7 co-expression has been reported as a characteristic of more advanced colorectal cancers (Hernandez *et al.*, 2005), whereas reduced K20 levels have been detected in association with high microsatellite instability (McGregor *et al.*, 2004). Pancreatic, biliary tract, esophageal and gastric adenocarcinomas uniformly express K7 and more variably, but up to 65%, K20 (Chu *et al.*, 2000), whereas a $K7^+/K20^-$ phenotype is characteristic of ovarian, endometrial and lung adenocarcinomas (Moll *et al.*, 2008). Endometrial adenocarcinomas may co-express stratified epithelial keratins, such as K5, as an indication of squamous metaplasia (Chu and Weiss, 2002a). Non-squamous, malignant salivary gland carcinomas are also $K7^+/K20^-$, with the exception of salivary duct carcinomas, which may be positive for both keratins (Nikitakis *et al.*, 2004). Furthermore, almost all thyroid tumors (follicular, papillary and medullary subtypes) and two-thirds of malignant mesothelioma cases are $K7^+/K20^-$. The latter tumors, in contrast to most adenocarcinomas, consistently express keratinocyte-type keratins, notably K5, and vimentin (Yaziji *et al.*, 2006). Appendiceal and lung carcinoids, adrenal cortical, prostatic and hepatocellular carcinomas are negative for both K7 and K20 (Chu and Weiss, 2002b).

Most breast adenocarcinomas, including both ductal and lobular subtypes, constitutively express K7, K8, K18 and K19. However, K8 exhibits a predominantly peripheral staining pattern in ductal carcinoma as compared to a ring-like, perinuclear pattern in lobular carcinoma (Lehr *et al.*, 2000). In poorly differentiated adenocarcinomas corresponding to the basal-like subtype as defined by microarray-based expression profiling of breast tumors (Sorlie *et al.*, 2001), keratins characteristic of the basal cells of stratified epithelium, such as K5/6, K14 and K17, are also expressed. More recently, phospho(Ser73)-K8 was identified as a possible biomarker for lower beclin1 expression, and thus defective autophagy status, in breast tumors (Kongara *et al.*, 2010).

Keratin expression is a particularly useful guide in the correct classification of renal cell carcinomas (RCCs) (Liu *et al.*, 2007), as clear-cell RCCs mainly express K8 and K18 with

minor K19 expression, papillary tumors strongly express K19 and K7 in addition to the basic K8/K18 pair and chromophobe RCCs typically express K7 and K8/K18, but little K19. Benign oncocytomas may histologically resemble chromophobe RCCs, but are K7 negative (Liu *et al.*, 2007). Transitional cell carcinomas generally conserve the urothelial keratin pattern showing combined expression of K8/K18, K7 and K19 together with K13 and K20 (Moll *et al.*, 1992).

Squamous cell carcinomas, independently of their site of origin, are characterized by the expression of the stratified epithelial keratins K5, K14 and K17 and the hyperproliferative keratinocyte-type keratins K6 and K16 (Moll *et al.*, 2008). K1/K10 may also be focally expressed, and K4 and K13 to a lesser extent. In poorly differentiated squamous cell carcinomas, co-expression of the simple epithelial keratins K8, K18 and K19 is often observed.

Use of keratins as diagnostic markers in tumor pathology is by far their most common application in the field of cancer. In cases remaining unclear on the basis of clinical presentation and conventional histopathology, including carcinomas that are poorly differentiated or spreading over several organs and metastases of unknown primary tumor site, keratin typing is especially valuable for correct tumor identification and subsequent selection of the most appropriate treatment plan.

Prognostic markers in epithelial tumors

Beyond their well-established role as diagnostic markers in cancer, keratins have also been recognized as prognostic indicators in a variety of epithelial malignancies (Table 2). For example, in colorectal cancer, reduced expression of K8 and K20 has been associated with epithelial-to-mesenchymal cancer cell transition, which is generally indicative of higher tumor aggressiveness, and decreased patient survival (Knosel *et al.*, 2006). Also, persistent or higher expression of a caspase-cleaved K18 fragment at Asp396 (produced by apoptotic epithelial cells and detected by an epitope-specific antibody M30) in the serum of colon cancer patients after primary tumor resection is indicative of systemic residual tumor load and significantly correlates with recurrence risk within 3 years (Ausch *et al.*, 2009). Higher serum-cleaved K18/M30 levels before treatment are also predictive of shorter survival in lung cancer patients (Ulukaya *et al.*, 2007). More recently, the ratio of caspase cleaved (M30) to total K18 (M65), conveniently determined in the serum or plasma using commercially available enzyme-linked immunosorbent assay kits, is being explored as a biomarker for therapy efficacy monitoring in carcinoma patients (Linder *et al.*, 2010). Similarly, in patients with intrahepatic cholangiocarcinoma, a high serum K19 fragment (CYFRA21-1) concentration is associated with decreased recurrence-free and overall survival (Uenishi *et al.*, 2008). Intratumoral K20 expression and K20 positivity in the bone marrow and/or blood correlate with worse prognosis in pancreatic adenocarcinomas (Soeth *et al.*, 2005; Matros *et al.*, 2006; Schmitz-Winnenthal *et al.*, 2006). Furthermore, in gastric cancer, real-time quantitative reverse transcription–polymerase chain reaction for K20 in peritoneal lavage fluid predicts peritoneal recurrence in patients undergoing resection with curative intent (Katsuragi *et al.*, 2007); K10 and K19 positivity in hepatocellular carcinomas are significant predictors of shorter overall and disease-free survival after surgical resection (Yang *et al.*, 2008); and absence of squamous differentation as evidenced by loss of K5/6 expression is associated with more aggressive endometrial carcinomas and reduced survival (Stefansson *et al.*, 2006). In clear-cell RCC, tumoral co-expression of K7 and K19 is associated with the lack of cytogenetic alterations, low nuclear grade and better clinical outcome (Mertz *et al.*, 2008), whereas detection of K8/18-positive circulating tumor cells correlates with positive lymph node status, presence of synchronous metastases at the time of primary tumor resection and poor overall survival in renal cell cancer (Bluemke *et al.*, 2009). Detection of disseminated keratin-positive tumor cells in the bone marrow of prostate

cancer patients before surgery is an independent risk factor for metastasis within 48 months (Weckermann *et al.*, 2009). In skin cancer, keratin expression in malignant melanoma is of particular interest, as K18 mRNA is surprisingly identified in one-third of melanoma tissue samples and is an adverse prognostic factor (Chen *et al.*, 2009).

In breast cancer, the molecularly defined basal-like subtype characterized by estrogen receptor (ER), progesterone receptor and human epidermal growth factor receptor-2 negativity, but epidermal growth factor receptor and K5/6 positivity, is associated with younger patient age, high tumor grade and poor prognosis, including shorter disease-free and overall survival (Cheang *et al.*, 2008; Yamamoto *et al.*, 2009). Expression of K17 in breast tumors is also prognostic of poor clinical outcome and this is independent of tumor size and grade in node-negative disease (van de Rijn *et al.*, 2002). Detection of K19 mRNA-positive circulating tumor cells before adjuvant chemotherapy predicts reduced disease-free and overall survival in patients with ER-negative, triple-negative and human epidermal growth factor receptor2-positive early breast tumors (Ignatiadis *et al.*, 2007), whereas the presence of K19 mRNA-positive circulating tumor cells in the blood after completion of adjuvant chemotherapy in women with early breast cancer of any subtype indicates the presence of chemotherapy-resistant residual disease and is again associated with higher risk of disease recurrence and decreased patient survival (Xenidis *et al.*, 2009). Gene expression profiling has indicated that K18 is frequently downregulated in metastatic breast cancer (Hedenfalk *et al.*, 2001; Zajchowski *et al.*, 2001), a finding associated with advanced tumor stage and grade, bone marrow micrometastasis, and shorter cancer-specific survival and overall survival (Woelfle *et al.*, 2003, 2004). Also, ubiquitin-immunoreactive degradation products of K8 and K18 are detected in breast carcinomas and may determine tumor aggressiveness (Iwaya *et al.*, 2003).

Functional role in tumorigenesis

Given their emerging regulatory role in normal cell physiology and their frequently altered expression in cancer, the question as to whether keratins play any functional role in epithelial tumorigenesis arises. Although most keratin KO and transgenic mice do not have any apparent tumor phenotype, K8 deficiency (in the FVB background) results in colorectal hyperplasia and inflammation (Baribault *et al.*, 1994; Habtezion *et al.*, 2005), and also affects (shortens) the latency, but not the incidence or the morphological features of polyoma middle T-induced mammary adenocarcinomas (Baribault *et al.*, 1997); human K8 overexpression results in early neoplastic-like alterations in the pancreas, including loss of acinar architecture, dysplasia and increased cell proliferation (Casanova *et al.*, 1999), and correlates with the extent of spontaneous pancreatic injury (Toivola *et al.*, 2008); and finally, ectopic expression of K8 in the skin causes epidermal hyperplasia in young mice, epidermal atypia and preneoplastic changes in aging mice, and malignant progression of benign skin tumors induced by chemical skin carcinogenesis assays (Casanova *et al.*, 2004).

Several studies have provided evidence supporting an active keratin role in cancer cell invasion and metastasis. Transfection of K8 and K18 in mouse L cells, which are fibroblasts and express vimentin, results in keratin filament formation and is associated with deformability and higher migratory and invasive abilities, indicating that keratins may influence cell shape and migration through interactions with the extracellular environment (Chu *et al.*, 1993). Similarly, experimental co-expression of vimentin and K8/K18 increases invasion and migration of human melanoma (Chu *et al.*, 1996) and breast cancer (Hendrix *et al.*, 1997) cells *in vitro*.

Incubation of human pancreatic cancer cells with sphingosylphosphorylcholine, a bioactive lipid present in high-density lipoprotein particles and found at increased levels in the blood and malignant ascites from ovarian cancer patients, induces keratin reorganization to a

perinuclear, ring-like structure, which is accompanied by K8 and K18 phosphorylation at Ser431 and Ser52, respectively (Beil *et al.*, 2003). This change in the keratin network architecture results in increased cellular elasticity and enhanced cell migration, indicating that sphingosylphosphorylcholine -induced keratin remodeling may directly contribute to the metastatic potential of epithelial cancer cells (Suresh *et al.*, 2005). Cell deformability is also increased in association with keratin network alterations owing to sphingosylphosphorylcholine, likely resulting in greater cancer cell ability to invade the surrounding tissue and permeate through the stroma, and thus facilitating its escape from the primary tumor (Rolli *et al.*, 2010). Furthermore, recent work has implicated alterations in keratin phosphorylation as a contributing factor to colorectal cancer progression, as K8 is a physiological substrate of phosphatase of regenerating liver-3, which is known to promote invasiveness and the metastatic potential of colorectal cancer cells, and high phosphatase of regenerating liver-3 levels are associated with reduction or loss of phosphorylated K8 at the invasive front of human colorectal cancer specimens and in liver metastases (Mizuuchi *et al.*, 2009).

Several studies have explored the role of keratins in cancer cell invasion by investigating K8-mediated plasminogen activation to the serine protease plasmin, which is involved in extracellular matrix remodeling and, as such, in tumor progression and metastasis. Plasminogen is activated on the cell surface by the urokinase-type plasminogen activator bound to urokinase-type plasminogen activator receptor and the C-terminal domain of K8 that penetrates the cellular membrane (K8 ectoplasmic domain), as shown in hepatocellular and breast carcinoma cells (Hembrough *et al.*, 1995). Although unlikely that keratin makes it to the cell surface through the regular secretory pathway (Riopel *et al.*, 1993), a monoclonal antibody to the K8 ectoplasmic domain prevents urokinase-type plasminogen activator binding and inhibits plasmin generation, which in turn results in altered cell morphology, greater cell adhesion to fibronectin and reduced breast cancer cell invasion potential (Obermajer *et al.*, 2009), indicating that K8 together with urokinase-type plasminogen activator, plasminogen and fibronectin form a signaling platform that can modulate cell adhesion and invasiveness of breast cancer cells.

K18 may play a regulatory role in hormonally responsive breast cancer, as it can effectively associate with and sequester the *ER*α target gene and ERα coactivator LRP16 in the cytoplasm, thus attenuating ERα-mediated signaling and estrogen-stimulated cell cycle progression in breast tumor cells (Meng *et al.*, 2009). Furthermore, autophagy defects, which promote mammary tumorigenesis (Karantza-Wadsworth *et al.*, 2007), result in K8, K17 and K19 upregulation in mouse mammary tumor cells under metabolic stress *in vitro* and in allograft mouse mammary tumors *in vivo* (Kongara *et al.*, 2010), potentially implicating deregulation of keratin homeostasis in defective autophagy-associated breast cancer, a hypothesis worthy of further investigation. Defective autophagy has also been implicated in abnormal keratin accumulation in the liver, as Mallory–Denk body-like inclusion formation, which is a common finding in hepatocellular carcinomas, is directly affected by pharmacological autophagy modulation (Harada *et al.*, 2008).

Keratin 17, which is rapidly induced in wounded stratified epithelia, regulates cell size and growth by binding to the adaptor protein 14-3-3σ and stimulating the mTOR pathway, thus regulating protein synthesis (Kim *et al.*, 2006). Additional evidence that keratins may function upstream of mTOR is provided by studies in mice with ablation of all keratin genes, where embryonic lethality from severe growth retardation is associated with aberrant localization of the glucose transporters GLUT1 and GLUT3m resulting in adenosine monophosphate kinase activation and suppression of the mTORC1 downstream targets S6 kinase and 4E-BP1 (Vijayaraj *et al.*, 2009). In an apparently reciprocal relationship, AKT isoforms regulate intermediate filament expression in epithelial cancer cell lines, as

overexpression of AKT1 increases K8/K18 levels and AKT2 upregulates K18 and vimentin (Fortier *et al.*, 2010). Thus, keratins, which are often aberrantly expressed in epithelial cancers, interact in multiple ways with the AKT/mTOR pathway, which itself is frequently abnormally activated in aggressive tumors, raising the possibility that the role of AKT in epithelial tumorigenesis is at least partially keratin mediated and/or dependent.

Keratins are also important for chaperone-mediated intracellular signaling, which may in turn play a role in epithelial tumorigenesis. Atypical PKC is an evolutionarily conserved key regulator of cellular asymmetry, which has also been identified as an oncogene causative of non-small-cell lung cancer and a predisposing factor for colon cancer, when overexpressed (Fields and Regala, 2007). Recent work showed that both filamentous keratins and heat-shock protein 70 are required for the rescue rephosphorylation of mature atypical PKC, thus regulating its subcellular distribution and maintaining its steady-state levels and activity (Mashukova *et al.*, 2009). Furthermore, given an excess of soluble heat-shock protein 70, the keratin network was expected to be a rate-limiting step in the atypical PKC rescue mechanism, a hypothesis confirmed in two different K8-overexpression animal models (Mashukova *et al.*, 2009). In both cases, cellular regions with abnormal and excessive intermediate filament accumulation also exhibited grossly mislocalized active atypical PKC signal, indicating that chaperone-assisted oncogenic kinase activity, including Akt1, may also depend on keratins and expanding on already available knowledge on the role of keratins as chaperone scaffolds (van den *et al.*, 1999; Toivola *et al.*, 2010).

Although K8 mutations have been implicated in the progression of acute and chronic (Ku *et al.*, 2001) liver disease, they have not been directly linked to hepatocellular, pancreatic (Treiber *et al.*, 2006) or any other carcinoma. To date, the only keratin and tumor type for which a specific variant or single-nucleotide polymorphism has been associated with cancer predisposition is K5 in basal cell carcinoma (Stacey *et al.*, 2009), as a genome-wide single-nucleotide polymorphism association scan for common basal cell carcinoma risk variants identified the G138E substitution in K5 as conferring susceptibility to basal cell carcinoma, but not to squamous cell carcinoma, cutaneous melanoma or fair-pigmentation traits. Given the increasing number of genome-wide association studies for different cancers, it is possible that additional keratin variants influencing specific cancer risk may be discovered in the near future.

Role in drug responsiveness

Keratins protect epithelial cells from mechanical stress, but also provide resistance to other cellular stressors that can lead to cell death, including death receptor activation and chemotherapeutic drugs. For example, K8- and K18-null mice, which lack keratin intermediate filaments in their hepatocytes owing to keratin instability when the partner keratin is missing, and hepatocytes cultured ex vivo from K8-null mice are more sensitive to Fas-mediated apoptosis than their wild-type counterparts (Gilbert *et al.*, 2001). Similarly, a K18 mutation (Arg89Cys) disrupting the keratin filament network predisposes hepatocytes to Fas- but not tumor necrosis factor-mediated apoptotic injury (Ku *et al.*, 2003b). These findings clearly show that K8 and K18 mediate resistance to Fas-induced apoptosis in the liver; however, they may also be relevant to cancer therapy, as keratin levels are affected by anticancer drugs, such as mitoxantrone (MX) (Cress *et al.*, 1988) and doxorubicin (Hammer *et al.*, 2010), and proapoptotic receptor agonists may have selective antitumor activity, as activation of the extrinsic apoptotic cell death pathway by binding of the apoptosis ligand 2/tumor necrosis factor-related apoptosis-inducing ligand to cognate death receptors results in apoptosis of different cancer cell types without significant toxicity toward normal cells (Ashkenazi, 2008; Gonzalvez and Ashkenazi, 2010).

Aberrant keratin expression has already been shown to confer a multidrug resistance phenotype, as mouse L fibroblasts are rendered resistant to MX, doxorubicin, methotrexate, melphalan and vincristine, but not to ionizing radiation, upon K8 and K18 transfection (Bauman *et al.*, 1994). Similarly, NIH 3T3 fibroblasts with ectopic K8/K18 expression exhibit resistance to MX, doxorubicin, bleomycin, mitomycin C and melphalan, but not to cisplatin (Anderson *et al.*, 1996). Furthermore, monocyte chemoattractant protein-7/MX, an MX-selected human breast cancer cell line with a multidrug resistance phenotype owing to overexpression of the breast cancer resistant protein, also exhibits elevated K8 levels, which synergize with breast cancer resistant protein in increasing drug resistance, likely acting via different mechanisms, as anti-K8 short hairpin RNA reverses MX resistance without promoting intracellular drug accumulation as breast cancer resistant protein knockdown does (Liu *et al.*, 2008b). The multidrug resistance of monocyte chemoattractant protein-7/MX cells is at least partially owing to their increased adhesion to the extracellular matrix, which is in turn mediated by K8 expression on the cell surface, indicating that alterations in the expression level and cellular localization of K8 may actively decrease response to cancer treatment (Liu *et al.*, 2008a). Whether pharmacological keratin modulation can be used as an adjunct to chemotherapy for improving therapeutic outcomes remains to be explored.

Concluding remarks

Keratins are important protectors of epithelial structural integrity under conditions of stress, but have also been recognized as regulators of other cellular functions, including motility, signaling, growth and protein synthesis. In cancer, keratins have traditionally been used as diagnostic tools, but accumulating evidence points to their importance as prognostic markers and, more interestingly, as active regulators of epithelial tumorigenesis and treatment responsiveness. Further investigation into the multifunctional role of keratins in cancer is warranted, and will hopefully result in the emergence of improved diagnostic and prognostic markers and the identification of novel therapeutic targets, in turn leading to earlier cancer detection and the rational design of more efficacious cancer therapies.

Acknowledgments

This work was supported by NIH Grant R00CA133181 and a Damon Runyon Clinical Investigator Award (VK).

References

Anderson JM, Heindl LM, Bauman PA, Ludi CW, Dalton WS, Cress AE. Cytokeratin expression results in a drug-resistant phenotype to six different chemotherapeutic agents. Clin Cancer Res. 1996; 2:97–105. [PubMed: 9816096]

Ashkenazi A. Directing cancer cells to self-destruct with proapoptotic receptor agonists. Nat Rev Drug Discov. 2008; 7:1001–1012. [PubMed: 18989337]

Ausch C, Buxhofer-Ausch V, Olszewski U, Hinterberger W, Ogris E, Schiessel R, et al. Caspase-cleaved cytokeratin 18 fragment (M30) as marker of postoperative residual tumor load in colon cancer patients. Eur J Surg Oncol. 2009; 35:1164–1168. [PubMed: 19254831]

Baribault H, Penner J, Iozzo RV, Wilson-Heiner M. Colorectal hyperplasia and inflammation in keratin 8-deficient FVB/N mice. Genes Dev. 1994; 8:2964–2973. [PubMed: 7528156]

Baribault H, Price J, Miyai K, Oshima RG. Mid-gestational lethality in mice lacking keratin 8. Genes Dev. 1993; 7:1191–1202. [PubMed: 7686525]

Baribault H, Wilson-Heiner M, Muller W, Penner J, Bakhiet N. Functional analysis of mouse keratin 8 in polyoma middle T-induced mammary gland tumours. Transgenic Res. 1997; 6:359–367. [PubMed: 9423286]

Bauman PA, Dalton WS, Anderson JM, Cress AE. Expression of cytokeratin confers multiple drug resistance. Proc Natl Acad Sci USA. 1994; 91:5311–5314. [PubMed: 7515497]

Beil M, Micoulet A, von Wichert G, Paschke S, Walther P, Omary MB, et al. Sphingosylphosphorylcholine regulates keratin network architecture and visco-elastic properties of human cancer cells. Nat Cell Biol. 2003; 5:803–811. [PubMed: 12942086]

Bluemke K, Bilkenroth U, Meye A, Fuessel S, Lautenschlaeger C, Goebel S, et al. Detection of circulating tumor cells in peripheral blood of patients with renal cell carcinoma correlates with prognosis. Cancer Epidemiol Biomarkers Prev. 2009; 18:2190–2194. [PubMed: 19661076]

Bonifas JM, Rothman AL, Epstein EH Jr. Epidermolysis bullosa simplex: evidence in two families for keratin gene abnormalities. Science. 1991; 254:1202–1205. [PubMed: 1720261]

Bragulla HH, Homberger DG. Structure and functions of keratin proteins in simple, stratified, keratinized and cornified epithelia. J Anat. 2009; 214:516–559. [PubMed: 19422428]

Buning C, Halangk J, Dignass A, Ockenga J, Deindl P, Nickel R, et al. Keratin 8 Y54H and G62C mutations are not associated with inflammatory bowel disease. Dig Liver Dis. 2004; 36:388–391. [PubMed: 15248378]

Casanova ML, Bravo A, Martinez-Palacio J, Fernandez-Acenero MJ, Villanueva C, Larcher F, et al. Epidermal abnormalities and increased malignancy of skin tumors in human epidermal keratin 8-expressing transgenic mice. FASEB J. 2004; 18:1556–1558. [PubMed: 15319370]

Casanova ML, Bravo A, Ramirez A, Morreale de Escobar G, Were F, Merlino G, et al. Exocrine pancreatic disorders in transsgenic mice expressing human keratin 8. J Clin Invest. 1999; 103:1587–1595. [PubMed: 10359568]

Cavestro GM, Frulloni L, Nouvenne A, Neri TM, Calore B, Ferri B, et al. Association of keratin 8 gene mutation with chronic pancreatitis. Dig Liver Dis. 2003; 35:416–420. [PubMed: 12868678]

Chan R, Rossitto PV, Edwards BF, Cardiff RD. Presence of proteolytically processed keratins in the culture medium of MCF-7 cells. Cancer Res. 1986; 46:6353–6359. [PubMed: 2430694]

Cheang MC, Voduc D, Bajdik C, Leung S, McKinney S, Chia SK, et al. Basal-like breast cancer defined by five biomarkers has superior prognostic value than triple-negative phenotype. Clin Cancer Res. 2008; 14:1368–1376. [PubMed: 18316557]

Chen N, Gong J, Chen X, Xu M, Huang Y, Wang L, et al. Cytokeratin expression in malignant melanoma: potential application of *in-situ* hybridization analysis of mRNA. Melanoma Res. 2009; 19:87–93. [PubMed: 19190520]

Chu P, Wu E, Weiss LM. Cytokeratin 7 and cytokeratin 20 expression in epithelial neoplasms: a survey of 435 cases. Mod Pathol. 2000; 13:962–972. [PubMed: 11007036]

Chu PG, Weiss LM. Expression of cytokeratin 5/6 in epithelial neoplasms: an immunohistochemical study of 509 cases. Mod Pathol. 2002a; 15:6–10. [PubMed: 11796835]

Chu PG, Weiss LM. Keratin expression in human tissues and neoplasms. Histopathology. 2002b; 40:403–439. [PubMed: 12010363]

Chu YW, Runyan RB, Oshima RG, Hendrix MJ. Expression of complete keratin filaments in mouse L cells augments cell migration and invasion. Proc Natl Acad Sci USA. 1993; 90:4261–4265. [PubMed: 7683431]

Chu YW, Seftor EA, Romer LH, Hendrix MJ. Experimental coexpression of vimentin and keratin intermediate filaments in human melanoma cells augments motility. Am J Pathol. 1996; 148:63–69. [PubMed: 8546227]

Coulombe PA, Hutton ME, Letai A, Hebert A, Paller AS, Fuchs E. Point mutations in human keratin 14 genes of epidermolysis bullosa simplex patients: genetic and functional analyses. Cell. 1991; 66:1301–1311. [PubMed: 1717157]

Coulombe PA, Omary MB. 'Hard' and 'soft' principles defining the structure, function and regulation of keratin intermediate filaments. Curr Opin Cell Biol. 2002; 14:110–122. [PubMed: 11792552]

Cress AE, Roberts RA, Bowden GT, Dalton WS. Modification of keratin by the chemotherapeutic drug mitoxantrone. Biochem Pharmacol. 1988; 37:3043–3046. [PubMed: 2456070]

Felder E, Siebenbrunner M, Busch T, Fois G, Miklavc P, Walther P, et al. Mechanical strain of alveolar type II cells in culture: changes in the transcellular cytokeratin network and adaptations. Am J Physiol Lung Cell Mol Physiol. 2008; 295:L849–L857. [PubMed: 18708634]

Fickert P, Trauner M, Fuchsbichler A, Stumptner C, Zatloukal K, Denk H. Mallory body formation in primary biliary cirrhosis is associated with increased amounts and abnormal phosphorylation and ubiquitination of cytokeratins. J Hepatol. 2003; 38:387–394. [PubMed: 12663227]

Fields AP, Regala RP. Protein kinase C iota: human oncogene, prognostic marker and therapeutic target. Pharmacol Res. 2007; 55:487–497. [PubMed: 17570678]

Fortier AM, Van Themsche C, Asselin E, Cadrin M. Akt isoforms regulate intermediate filament protein levels in epithelial carcinoma cells. FEBS Lett. 2010; 584:984–988. [PubMed: 20109457]

Fuchs E, Cleveland DW. A structural scaffolding of intermediate filaments in health and disease. Science. 1998; 279:514–519. [PubMed: 9438837]

Gilbert S, Loranger A, Daigle N, Marceau N. Simple epithelium keratins 8 and 18 provide resistance to Fas-mediated apoptosis. The protection occurs through a receptor-targeting modulation. J Cell Biol. 2001; 154:763–773. [PubMed: 11514590]

Glotzer M. The 3Ms of central spindle assembly: microtubules, motors and MAPs. Nat Rev Mol Cell Biol. 2009; 10:9–20. [PubMed: 19197328]

Gonzalvez F, Ashkenazi A. New insights into apoptosis signaling by Apo2L/TRAIL. Oncogene. 2010; 29:4752–4765. [PubMed: 20531300]

Habtezion A, Toivola DM, Butcher EC, Omary MB. Keratin-8-deficient mice develop chronic spontaneous Th2 colitis amenable to antibiotic treatment. J Cell Sci. 2005; 118:1971–1980. [PubMed: 15840656]

Hammer E, Bien S, Salazar MG, Steil L, Scharf C, Hildebrandt P, et al. Proteomic analysis of doxorubicin-induced changes in the proteome of HepG2cells combining 2-D DIGE and LC-MS/MS approaches. Proteomics. 2010; 10:99–114. [PubMed: 20017144]

Harada M, Strnad P, Toivola DM, Omary MB. Autophagy modulates keratin-containing inclusion formation and apoptosis in cell culture in a context-dependent fashion. Exp Cell Res. 2008; 314:1753–1764. [PubMed: 18343366]

He T, Stepulak A, Holmstrom TH, Omary MB, Eriksson JE. The intermediate filament protein keratin 8 is a novel cytoplasmic substrate for c-Jun N-terminal kinase. J Biol Chem. 2002; 277:10767–10774. [PubMed: 11781324]

Hedenfalk I, Duggan D, Chen Y, Radmacher M, Bittner M, Simon R, et al. Gene-expression profiles in hereditary breast cancer. N Engl J Med. 2001; 344:539–548. [PubMed: 11207349]

Hembrough TA, Vasudevan J, Allietta MM, Glass WF II, Gonias SL. A cytokeratin 8-like protein with plasminogen-binding activity is present on the external surfaces of hepatocytes, HepG2 cells and breast carcinoma cell lines. J Cell Sci. 1995; 108(Part 3):1071–1082. [PubMed: 7542667]

Hendrix MJ, Seftor EA, Seftor RE, Trevor KT. Experimental co-expression of vimentin and keratin intermediate filaments in human breast cancer cells results in phenotypic interconversion and increased invasive behavior. Am J Pathol. 1997; 150:483–495. [PubMed: 9033265]

Herrmann H, Bar H, Kreplak L, Strelkov SV, Aebi U. Intermediate filaments: from cell architecture to nanomechanics. Nat Rev Mol Cell Biol. 2007; 8:562–573. [PubMed: 17551517]

Herrmann H, Strelkov SV, Burkhard P, Aebi U. Intermediate filaments: primary determinants of cell architecture and plasticity. J Clin Invest. 2009; 119:1772–1783. [PubMed: 19587452]

Hernandez BY, Frierson HF, Moskaluk CA, Li YJ, Clegg L, Cote TR, et al. CK20 and CK7 protein expression in colorectal cancer: demonstration of the utility of a population-based tissue microarray. Hum Pathol. 2005; 36:275–281. [PubMed: 15791572]

Ignatiadis M, Xenidis N, Perraki M, Apostolaki S, Politaki E, Kafousi M, et al. Different prognostic value of cytokeratin-19 mRNA positive circulating tumor cells according to estrogen receptor and HER2 status in early-stage breast cancer. J Clin Oncol. 2007; 25:5194–5202. [PubMed: 17954712]

Irvine AD, Corden LD, Swensson O, Swensson B, Moore JE, Frazer DG, et al. Mutations in cornea-specific keratin K3 or K12 genes cause Meesmann's corneal dystrophy. Nat Genet. 1997; 16:184–187. [PubMed: 9171831]

Iwaya K, Ogawa H, Mukai Y, Iwamatsu A, Mukai K. Ubiquitin-immunoreactive degradation products of cytokeratin 8/18 correlate with aggressive breast cancer. Cancer Sci. 2003; 94:864–870. [PubMed: 14556659]

Jaitovich A, Mehta S, Na N, Ciechanover A, Goldman RD, Ridge KM. Ubiquitin-proteasome-mediated degradation of keratin intermediate filaments in mechanically stimulated A549 cells. J Biol Chem. 2008; 283:25348–25355. [PubMed: 18617517]

Kao WW, Liu CY, Converse RL, Shiraishi A, Kao CW, Ishizaki M, et al. Keratin 12-deficient mice have fragile corneal epithelia. Invest Ophthalmol Vis Sci. 1996; 37:2572–2584. [PubMed: 8977471]

Karantza-Wadsworth V, Patel S, Kravchuk O, Chen G, Mathew R, Jin S, et al. Autophagy mitigates metabolic stress and genome damage in mammary tumorigenesis. Genes Dev. 2007; 21:1621–1635. [PubMed: 17606641]

Karantza-Wadsworth V, White E. A mouse mammary epithelial cell model to identify molecular mechanisms regulating breast cancer progression. Methods Enzymol. 2008; 446:61–76. [PubMed: 18603116]

Katsuragi K, Yashiro M, Sawada T, Osaka H, Ohira M, Hirakawa K. Prognostic impact of PCR-based identification of isolated tumour cells in the peritoneal lavage fluid of gastric cancer patients who underwent a curative R0 resection. Br J Cancer. 2007; 97:550–556. [PubMed: 17667927]

Kim S, Wong P, Coulombe PA. A keratin cytoskeletal protein regulates protein synthesis and epithelial cell growth. Nature. 2006; 441:362–365. [PubMed: 16710422]

Knosel T, Emde V, Schluns K, Schlag PM, Dietel M, Petersen I. Cytokeratin profiles identify diagnostic signatures in colorectal cancer using multiplex analysis of tissue microarrays. Cell Oncol. 2006; 28:167–175. [PubMed: 16988472]

Kongara S, Kravchuk O, Teplova I, Lozy F, Schulte J, Moore D, et al. Autophagy regulates keratin 8 homeostasis in mammary epithelial cells and in breast tumors. Mol Cancer Res. 2010; 8:873–884. [PubMed: 20530580]

Ku NO, Azhar S, Omary MB. Keratin 8 phosphorylation by p38 kinase regulates cellular keratin filament reorganization: modulation by a keratin 1-like disease causing mutation. J Biol Chem. 2002a; 277:10775–10782. [PubMed: 11788583]

Ku NO, Darling JM, Krams SM, Esquivel CO, Keeffe EB, Sibley RK, et al. Keratin 8 and 18 mutations are risk factors for developing liver disease of multiple etiologies. Proc Natl Acad Sci USA. 2003a; 100:6063–6068. [PubMed: 12724528]

Ku NO, Gish R, Wright TL, Omary MB. Keratin 8 mutations in patients with cryptogenic liver disease. N Engl J Med. 2001; 344:1580–1587. [PubMed: 11372009]

Ku NO, Michie S, Oshima RG, Omary MB. Chronic hepatitis, hepatocyte fragility, and increased soluble phosphoglycokeratins in transgenic mice expressing a keratin 18 conserved arginine mutant. J Cell Biol. 1995; 131:1303–1314. [PubMed: 8522591]

Ku NO, Michie S, Resurreccion EZ, Broome RL, Omary MB. Keratin binding to 14-3-3 proteins modulates keratin filaments and hepatocyte mitotic progression. Proc Natl Acad Sci USA. 2002b; 99:4373–4378. [PubMed: 11917136]

Ku NO, Omary MB. Keratins turn over by ubiquitination in a phosphorylation-modulated fashion. J Cell Biol. 2000; 149:547–552. [PubMed: 10791969]

Ku NO, Omary MB. A disease- and phosphorylation-related nonmechanical function for keratin 8. J Cell Biol. 2006; 174:115–125. [PubMed: 16818723]

Ku NO, Soetikno RM, Omary MB. Keratin mutation in transgenic mice predisposes to Fas but not TNF-induced apoptosis and massive liver injury. Hepatology. 2003b; 37:1006–1014. [PubMed: 12717381]

Ku NO, Strnad P, Zhong BH, Tao GZ, Omary MB. Keratins let liver live: mutations predispose to liver disease and crosslinking generates Mallory–Denk bodies. Hepatology. 2007; 46:1639–1649. [PubMed: 17969036]

Ku NO, Wright TL, Terrault NA, Gish R, Omary MB. Mutation of human keratin 18 in association with cryptogenic cirrhosis. J Clin Invest. 1997; 99:19–23. [PubMed: 9011570]

Ku NO, Zhou X, Toivola DM, Omary MB. The cytoskeleton of digestive epithelia in health and disease. Am J Physiol. 1999; 277:G1108–G1137. [PubMed: 10600809]

Lane EB, McLean WH. Keratins and skin disorders. J Pathol. 2004; 204:355–366. [PubMed: 15495218]

Lane EB, Rugg EL, Navsaria H, Leigh IM, Heagerty AH, Ishida-Yamamoto A, et al. A mutation in the conserved helix termination peptide of keratin 5 in hereditary skin blistering. Nature. 1992; 356:244–246. [PubMed: 1372711]

Lehr HA, Folpe A, Yaziji H, Kommoss F, Gown AM. Cytokeratin 8 immunostaining pattern and E-cadherin expression distinguish lobular from ductal breast carcinoma. Am J Clin Pathol. 2000; 114:190–196. [PubMed: 10941333]

Liao J, Ku NO, Omary MB. Stress, apoptosis, and mitosis induce phosphorylation of human keratin 8 at Ser-73 in tissues and cultured cells. J Biol Chem. 1997; 272:17565–17573. [PubMed: 9211903]

Linder S, Olofsson MH, Herrmann R, Ulukaya E. Utilization of cytokeratin-based biomarkers for pharmacodynamic studies. Expert Rev Mol Diagn. 2010; 10:353–359. [PubMed: 20370591]

Liu F, Chen Z, Wang J, Shao X, Cui Z, Yang C, et al. Overexpression of cell surface cytokeratin 8 in multidrug-resistant MCF-7/MX cells enhances cell adhesion to the extracellular matrix. Neoplasia. 2008a; 10:1275–1284. [PubMed: 18953437]

Liu F, Fan D, Qi J, Zhu H, Zhou Y, Yang C, et al. Co-expression of cytokeratin 8 and breast cancer resistant protein indicates a multifactorial drug-resistant phenotype in human breast cancer cell line. Life Sci. 2008b; 83:496–501. [PubMed: 18725232]

Liu L, Qian J, Singh H, Meiers I, Zhou X, Bostwick DG. Immunohistochemical analysis of chromophobe renal cell carcinoma, renal oncocytoma, and clear cell carcinoma: an optimal and practical panel for differential diagnosis. Arch Pathol Lab Med. 2007; 131:1290–1297. [PubMed: 17683191]

Lloyd C, Yu QC, Cheng J, Turksen K, Degenstein L, Hutton E, et al. The basal keratin network of stratified squamous epithelia: defining K15 function in the absence of K14. J Cell Biol. 1995; 129:1329–1344. [PubMed: 7539810]

Loranger A, Duclos S, Grenier A, Price J, Wilson-Heiner M, Baribault H. Simple epithelium keratins are required for maintenance of hepatocyte integrity. Am J Pathol. 1997; 151:1673–1683. [PubMed: 9403718]

Magin TM, Schroder R, Leitgeb S, Wanninger F, Zatloukal K, Grund C, et al. Lessons from keratin 18 knockout mice: formation of novel keratin filaments, secondary loss of keratin 7 and accumulation of liver-specific keratin 8-positive aggregates. J Cell Biol. 1998; 140:1441–1451. [PubMed: 9508776]

Mashukova A, Oriolo AS, Wald FA, Casanova ML, Kroger C, Magin TM, et al. Rescue of atypical protein kinase C in epithelia by the cytoskeleton and Hsp70 family chaperones. J Cell Sci. 2009; 122:2491–2503. [PubMed: 19549684]

Matros E, Bailey G, Clancy T, Zinner M, Ashley S, Whang E, et al. Cytokeratin 20 expression identifies a subtype of pancreatic adenocarcinoma with decreased overall survival. Cancer. 2006; 106:693–702. [PubMed: 16362976]

McGregor DK, Wu TT, Rashid A, Luthra R, Hamilton SR. Reduced expression of cytokeratin 20 in colorectal carcinomas with high levels of microsatellite instability. Am J Surg Pathol. 2004; 28:712–718. [PubMed: 15166663]

Meng Y, Wu Z, Yin X, Zhao Y, Chen M, Si Y, et al. Keratin 18 attenuates estrogen receptor alpha-mediated signaling by sequestering LRP16 in cytoplasm. BMC Cell Biol. 2009; 10:96. [PubMed: 20035625]

Mertz KD, Demichelis F, Sboner A, Hirsch MS, Dal Cin P, Struckmann K, et al. Association of cytokeratin 7 and 19 expression with genomic stability and favorable prognosis in clear cell renal cell cancer. Int J Cancer. 2008; 123:569–576. [PubMed: 18478571]

Mizuuchi E, Semba S, Kodama Y, Yokozaki H, et al. Down-modulation of keratin 8 phosphorylation levels by PRL-3 contributes to colorectal carcinoma progression. Int J Cancer. 2009; 124:1802–1810. [PubMed: 19115206]

Moll R, Divo M, Langbein L. The human keratins: biology and pathology. Histochem Cell Biol. 2008; 129:705–733. [PubMed: 18461349]

Moll R, Franke WW, Schiller DL, Geiger B, Krepler R. The catalog of human cytokeratins: patterns of expression in normal epithelia, tumors and cultured cells. Cell. 1982; 31:11–24. [PubMed: 6186379]

Moll R, Lowe A, Laufer J, Franke WW. Cytokeratin 20 in human carcinomas. A new histodiagnostic marker detected by monoclonal antibodies. Am J Pathol. 1992; 140:427–447. [PubMed: 1371204]

Moll R, Schiller DL, Franke WW. Identification of protein IT of the intestinal cytoskeleton as a novel type I cytokeratin with unusual properties and expression patterns. J Cell Biol. 1990; 111:567–580. [PubMed: 1696264]

Na N, Chandel NS, Litvan J, Ridge KM. Mitochondrial reactive oxygen species are required for hypoxia-induced degradation of keratin intermediate filaments. FASEB J. 2010; 24:799–809. [PubMed: 19897662]

Nelson DA, Tan TT, Rabson AB, Anderson D, Degenhardt K, White E. Hypoxia and defective apoptosis drive genomic instability and tumorigenesis. Genes Dev. 2004; 18:2095–2107. [PubMed: 15314031]

Nikitakis NG, Tosios KI, Papanikolaou VS, Rivera H, Papanicolaou SI, Ioffe OB. Immunohistochemical expression of cytokeratins 7 and 20 in malignant salivary gland tumors. Mod Pathol. 2004; 17:407–415. [PubMed: 14976534]

Obermajer N, Doljak B, Kos J. Cytokeratin 8 ectoplasmic domain binds urokinase-type plasminogen activator to breast tumor cells and modulates their adhesion, growth and invasiveness. Mol Cancer. 2009; 8:88. [PubMed: 19845941]

Omary MB, Coulombe PA, McLean WH. Intermediate filament proteins and their associated diseases. N Engl J Med. 2004; 351:2087–2100. [PubMed: 15537907]

Omary MB, Ku NO, Liao J, Price D. Keratin modifications and solubility properties in epithelial cells and *in vitro*. Subcell Biochem. 1998; 31:105–140. [PubMed: 9932491]

Omary MB, Ku NO, Strnad P, Hanada S. Toward unraveling the complexity of simple epithelial keratins in human disease. J Clin Invest. 2009; 119:1794–1805. [PubMed: 19587454]

Omary MB, Ku NO, Tao GZ, Toivola DM, Liao J. 'Heads and tails' of intermediate filament phosphorylation: multiple sites and functional insights. Trends Biochem Sci. 2006; 31:383–394. [PubMed: 16782342]

Oriolo AS, Wald FA, Ramsauer VP, Salas PJ. Intermediate filaments: a role in epithelial polarity. Exp Cell Res. 2007; 313:2255–2264. [PubMed: 17425955]

Peters B, Kirfel J, Bussow H, Vidal M, Magin TM. Complete cytolysis and neonatal lethality in keratin 5 knockout mice reveal its fundamental role in skin integrity and in epidermolysis bullosa simplex. Mol Biol Cell. 2001; 12:1775–1789. [PubMed: 11408584]

Pollard TD, Cooper JA. Actin, a central player in cell shape and movement. Science. 2009; 326:1208–1212. [PubMed: 19965462]

Quinlan RA, Cohlberg JA, Schiller DL, Hatzfeld M, Franke WW. Heterotypic tetramer (A2D2) complexes of non-epidermal keratins isolated from cytoskeletons of rat hepatocytes and hepatoma cells. J Mol Biol. 1984; 178:365–388. [PubMed: 6208369]

Ridge KM, Linz L, Flitney FW, Kuczmarski ER, Chou YH, Omary MB, et al. Keratin 8 phosphorylation by protein kinase C delta regulates shear stress-mediated disassembly of keratin intermediate filaments in alveolar epithelial cells. J Biol Chem. 2005; 280:30400–30405. [PubMed: 15972820]

Riopel CL, Butt I, Omary MB. Method of cell handling affects leakiness of cell surface labeling and detection of intracellular keratins. Cell Motil Cytoskeleton. 1993; 26:77–87. [PubMed: 7693356]

Rolli CG, Seufferlein T, Kemkemer R, Spatz JP. Impact of tumor cell cytoskeleton organization on invasiveness and migration: a microchannel-based approach. PLoS One. 2010; 5:e8726. [PubMed: 20090950]

Schmitz-Winnenthal FH, Volk C, Helmke B, Berger S, Hinz U, Koch M, et al. Expression of cytokeratin-20 in pancreatic cancer: an indicator of poor outcome after R0 resection. Surgery. 2006; 139:104–108. [PubMed: 16364723]

Schutte B, Henfling M, Kolgen W, Bouman M, Meex S, Leers MP, et al. Keratin 8/18 breakdown and reorganization during apoptosis. Exp Cell Res. 2004; 297:11–26. [PubMed: 15194421]

Schweizer J, Bowden PE, Coulombe PA, Langbein L, Lane EB, Magin TM, et al. New consensus nomenclature for mammalian keratins. J Cell Biol. 2006; 174:169–174. [PubMed: 16831889]

Sivaramakrishnan S, DeGiulio JV, Lorand L, Goldman RD, Ridge KM. Micromechanical properties of keratin intermediate filament networks. Proc Natl Acad Sci USA. 2008; 105:889–894. [PubMed: 18199836]

Sivaramakrishnan S, Schneider JL, Sitikov A, Goldman RD, Ridge KM. Shear stress induced reorganization of the keratin intermediate filament network requires phosphorylation by protein kinase C zeta. Mol Biol Cell. 2009; 20:2755–2765. [PubMed: 19357195]

Smith F. The molecular genetics of keratin disorders. Am J Clin Dermatol. 2003; 4:347–364. [PubMed: 12688839]

Soeth E, Grigoleit U, Moellmann B, Roder C, Schniewind B, Kremer B, et al. Detection of tumor cell dissemination in pancreatic ductal carcinoma patients by CK 20 RT-PCR indicates poor survival. J Cancer Res Clin Oncol. 2005; 131:669–676. [PubMed: 16136352]

Sorlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci USA. 2001; 98:10869–10874. [PubMed: 11553815]

Stacey SN, Sulem P, Masson G, Gudjonsson SA, Thorleifsson G, Jakobsdottir M, et al. New common variants affecting susceptibility to basal cell carcinoma. Nat Genet. 2009; 41:909–914. [PubMed: 19578363]

Stefansson IM, Salvesen HB, Akslen LA. Loss of p63 and cytokeratin 5/6 expression is associated with more aggressive tumors in endometrial carcinoma patients. Int J Cancer. 2006; 118:1227–1233. [PubMed: 16152605]

Steinert PM, Marekov LN, Parry DA. Conservation of the structure of keratin intermediate filaments: molecular mechanism by which different keratin molecules integrate into preexisting keratin intermediate filaments during differentiation. Biochemistry. 1993; 32:10046–10056. [PubMed: 7691168]

Strnad P, Zhou Q, Hanada S, Lazzeroni LC, Zhong BH, So P, et al. Keratin variants predispose to acute liver failure and adverse outcome: race and ethnic associations. Gastroenterology. 2010; 139:828–835. 835 e1–e3. [PubMed: 20538000]

Styers ML, Kowalczyk AP, Faundez V. Intermediate filaments and vesicular membrane traffic: the odd couple's first dance? Traffic. 2005; 6:359–365. [PubMed: 15813746]

Suresh S, Spatz J, Mills JP, Micoulet A, Dao M, Lim CT, et al. Connections between single-cell biomechanics and human disease states: gastrointestinal cancer and malaria. Acta Biomater. 2005; 1:15–30. [PubMed: 16701777]

Takahashi K, Paladini RD, Coulombe PA x. Cloning and characterization of multiple human genes and cDNAs encoding highly related type II keratin 6 isoforms. J Biol Chem. 1995; 270:18581–18592. [PubMed: 7543104]

Toivola DM, Ku NO, Resurreccion EZ, Nelson DR, Wright TL, Omary MB. Keratin 8 and 18 hyperphosphorylation is a marker of progression of human liver disease. Hepatology. 2004; 40:459–466. [PubMed: 15368451]

Toivola DM, Nakamichi I, Strnad P, Michie SA, Ghori N, Harada M, et al. Keratin overexpression levels correlate with the extent of spontaneous pancreatic injury. Am J Pathol. 2008; 172:882–892. [PubMed: 18349119]

Toivola DM, Strnad P, Habtezion A, Omary MB. Intermediate filaments take the heat as stress proteins. Trends Cell Biol. 2010; 20:79–91. [PubMed: 20045331]

Toivola DM, Tao GZ, Habtezion A, Liao J, Omary MB. Cellular integrity plus: organelle-related and protein-targeting functions of intermediate filaments. Trends Cell Biol. 2005; 15:608–617. [PubMed: 16202602]

Toivola DM, Zhou Q, English LS, Omary MB. Type II keratins are phosphorylated on a unique motif during stress and mitosis in tissues and cultured cells. Mol Biol Cell. 2002; 13:1857–1870. [PubMed: 12058054]

Treiber M, Schulz HU, Landt O, Drenth JP, Castellani C, Real FX, et al. Keratin 8 sequence variants in patients with pancreatitis and pancreatic cancer. J Mol Med. 2006; 84:1015–1022. [PubMed: 17039343]

Uenishi T, Yamazaki O, Tanaka H, Takemura S, Yamamoto T, Tanaka S, et al. Serum cytokeratin 19 fragment (CYFRA21-1) as a prognostic factor in intrahepatic cholangiocarcinoma. Ann Surg Oncol. 2008; 15:583–589. [PubMed: 17955299]

Ulukaya E, Yilmaztepe A, Akgoz S, Linder S, Karadag M. The levels of caspase-cleaved cytokeratin 18 are elevated in serum from patients with lung cancer and helpful to predict the survival. Lung Cancer. 2007; 56:399–404. [PubMed: 17316892]

van den IP, Norman DG, Quinlan RA. Molecular chaperones: small heat shock proteins in the limelight. Curr Biol. 1999; 9:R103–R105. [PubMed: 10021375]

van de Rijn M, Perou CM, Tibshirani R, Haas P, Kallioniemi O, Kononen J, et al. Expression of cytokeratins 17 and 5 identifies a group of breast carcinomas with poor clinical outcome. Am J Pathol. 2002; 161:1991–1996. [PubMed: 12466114]

Vijayaraj P, Kroger C, Reuter U, Windoffer R, Leube RE, Magin TM. Keratins regulate protein biosynthesis through localization of GLUT1 and -3 upstream of AMP kinase and Raptor. J Cell Biol. 2009; 187:175–184. [PubMed: 19841136]

Vijayaraj P, Sohl G, Magin TM. Keratin transgenic and knockout mice: functional analysis and validation of disease-causing mutations. Methods Mol Biol. 2007; 360:203–251. [PubMed: 17172732]

Wang J, Chan JY, Fong CC, Tzang CH, Fung KP, Yang M. Transcriptional analysis of doxorubicin-induced cytotoxicity and resistance in human hepatocellular carcinoma cell lines. Liver Int. 2009; 29:1338–1347. [PubMed: 19627484]

Weckermann D, Polzer B, Ragg T, Blana A, Schlimok G, Arnholdt H, et al. Perioperative activation of disseminated tumor cells in bone marrow of patients with prostate cancer. J Clin Oncol. 2009; 27:1549–1556. [PubMed: 19237635]

Winter H, Langbein L, Praetzel S, Jacobs M, Rogers MA, Leigh IM, et al. A novel human type II cytokeratin, K6hf, specifically expressed in the companion layer of the hair follicle. J Invest Dermatol. 1998; 111:955–962. [PubMed: 9856802]

Woelfle U, Cloos J, Sauter G, Riethdorf L, Janicke F, van Diest P. Molecular signature associated with bone marrow micrometastasis in human breast cancer. Cancer Res. 2003; 63:5679–5684. [PubMed: 14522883]

Woelfle U, Sauter G, Santjer S, Brakenhoff R, Pantel K. Down-regulated expression of cytokeratin 18 promotes progression of human breast cancer. Clin Cancer Res. 2004; 10:2670–2674. [PubMed: 15102669]

Woll S, Windoffer R, Leube RE. p38 MAPK-dependent shaping of the keratin cytoskeleton in cultured cells. J Cell Biol. 2007; 177:795–807. [PubMed: 17535969]

Xenidis N, Ignatiadis M, Apostolaki S, Perraki M, Kalbakis K, Agelaki S. Cytokeratin-19 mRNA-positive circulating tumor cells after adjuvant chemotherapy in patients with early breast cancer. J Clin Oncol. 2009; 27:2177–2184. [PubMed: 19332733]

Yamamoto Y, Ibusuki M, Nakano M, Kawasoe T, Hiki R, Iwase H. Clinical significance of basal-like subtype in triple-negative breast cancer. Breast Cancer. 2009; 16:260–267. [PubMed: 19701681]

Yang XR, Xu Y, Shi GM, Fan J, Zhou J, Ji Y, et al. Cytokeratin 10 and cytokeratin 19: predictive markers for poor prognosis in hepatocellular carcinoma patients after curative resection. Clin Cancer Res. 2008; 14:3850–3859. [PubMed: 18559605]

Yano S, Komine M, Fujimoto M, Okochi H, Tamaki K. Mechanical stretching *in vitro* regulates signal transduction pathways and cellular proliferation in human epidermal keratinocytes. J Invest Dermatol. 2004; 122:783–790. [PubMed: 15086566]

Yaziji H, Battifora H, Barry TS, Hwang HC, Bacchi CE, McIntosh MW, et al. Evaluation of 12 antibodies for distinguishing epithelioid mesothelioma from adenocarcinoma: identification of a three-antibody immunohistochemical panel with maximal sensitivity and specificity. Mod Pathol. 2006; 19:514–523. [PubMed: 16554731]

Zajchowski DA, Bartholdi MF, Gong Y, Webster L, Liu HL, Munishkin A, et al. Identification of gene expression profiles that predict the aggressive behavior of breast cancer cells. Cancer Res. 2001; 61:5168–5178. [PubMed: 11431356]

Zatloukal K, French SW, Stumptner C, Strnad P, Harada M, Toivola DM, et al. From Mallory to Mallory–Denk bodies: what, how and why? Exp Cell Res. 2007; 313:2033–2049. [PubMed: 17531973]

Zatloukal K, Stumptner C, Lehner M, Denk H, Baribault H, Eshkind LG, et al. Cytokeratin 8 protects from hepatotoxicity, and its ratio to cytokeratin 18 determines the ability of hepatocytes to form Mallory bodies. Am J Pathol. 2000; 156:1263–1274. [PubMed: 10751352]

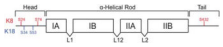

Figure 1.

Keratin structure. Keratins form obligate heteropolymers (between one type I and one type II keratin) and share a common structure consisting of a central coiled-coil α-helical rod domain that is flanked by non-helical head and tail domains. The α-helical rod domain is subdivided into four subdomains (coils IA, IB, IIA and IIB), which are connected with three linkers (L1, L12 and L2). Most post-translationally modified sites are found within the head and tail domains (conserved phosphorylation sites on human K8 and K18 are shown).

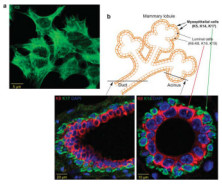

Figure 2.

Keratins in mammary epithelial cells *in vitro* and *in vivo*. (**a**) K8 immune staining in immortalized mouse mammary epithelial cells (Karantza-Wadsworth and White, 2008) in two-dimensional culture. (**b**) The mammary gland is a compound tubuloalveolar epithelial cell structure consisting of secretory acini grouped within lobules and draining into intralobular ducts, which in turn drain into interlobular ducts. Lobules are organized into 15–20 lobes emptying into lactiferous sinuses and from there into lactiferous ducts, which open onto the nipple. Ducts (left panel) and acini (right panel) are lined with luminal epithelial cells expressing K8/K18, K19 and K6. These are surrounded by myoepithelial cells, which are characterized by K5, K14 and K17 expression.

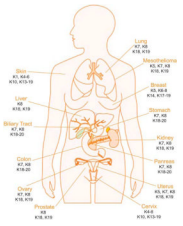

Figure 3.

Keratin expression in human cancer. Keratins are normally expressed in a cell type-, differentiation- and functional status-dependent manner, and epithelial cancers largely maintain the characteristics of keratin expression associated with their respective cell type of origin, so keratins have long been recognized as diagnostic markers in tumor pathology. Examples of keratins commonly used in the diagnosis of human epithelial malignancies are presented here.

Table 1

Keratins as diagnostic markers in tumor pathology

Cancer site and subtype	Keratin expression
Biliary duct	K7, K8, K18–20
Bladder, transitional cell	K5[a], K7, K8, K18, K19, K20[a]
Breast	K5[a,b], K6[a,b], K7, K8, K14[a,b], K17[a,b], K18, K19
Cervix	K4–8, K10, K13–19
Colon	K7[a], K8, K18–20
Kidney, clear cell	K8, K18, K19[a]
Papillary	K7, K8, K18, K19
Chromophobe	K7, K8, K18, K19[a]
Liver	K7[a], K8, K18, K19[a], K20[a]
Lung, adenocarcinoma	K7, K8, K18, K19
Small cell	K8, K18, K19[a]
Ovary, adenocarcinoma[c]	K7, K8, K18, K19
Pancreas	K5[a], K7, K8, K18, K19, K20[a]
Pleura (mesothelioma)	K5, K7[a], K8, K18, K19
Prostate	K8, K18, K19
Skin, squamous	K1, K4–6, K8[d], K10, K13–17, K18[d], K19[d]
Merkel cell	K8, K18, K20
Stomach	K7[a], K8, K18, K19, K20[a]
Uterus	K5[a], K7, K8, K18, K19

[a] Focal/heterogeneous staining in some, but not all, cases.

[b] Focal or extended staining in basal-like tumors.

[c] Non-mucinous.

[d] In poorly differentiated cases.

Table 2

Keratins as prognostic markers in tumor pathology

Cancer site	Keratin expression pattern	Detection site	Prognosis
Biliary duct	High K19 fragment (CYFRA21-1)	Serum	Worse
Breast	K5/6, K17	Tumor	Worse
	K19 mRNA	CTCs[a]	Worse
	Reduced K18 mRNA	Tumor	Worse
	Ubiquitinated K8 and K18 fragments	Tumor	Worse
Colon	Reduced K8, K20	Tumor	Worse
	Persistent or higher K18 fragment (M30) after primary tumor resection	Serum	Worse
Kidney	K8, K18	CTCs[a]	Worse
	K7, K19	Tumor	Better
Liver	K10, K19	Tumor	Worse
Lung	High K18 fragment (M30)	Serum	Worse
Pancreas	K20	Tumor	Worse
	K20	Serum	Worse
Prostate	K8, K18, K19 before surgery	Bone marrow	Worse
Skin (melanoma)	K18	Tumor	Worse
Stomach	K20	Peritoneal fluid	Worse
Uterus	Loss of K5/K6	Tumor	Worse

[a]Circulating tumor cells.