Review

Biological Functions of Cytokeratin 18 in Cancer

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Abstract

The structural proteins cytokeratin 18 (CK18) and its coexpressed complementary partner CK8 are expressed in a variety of adult epithelial organs and may play a role in carcinogenesis. In this study, we focused on the biological functions of CK18, which is thought to modulate intracellular signaling and operates in conjunction with various related proteins. CK18 may affect carcinogenesis through several signaling pathways, including the phosphoinositide 3-kinase (PI3K)/Akt, Wnt, and extracellular signal-regulated kinase (ERK) mitogen-activated protein kinase (MAPK) signaling pathways. CK18 acts as an identical target of Akt in the PI3K/Akt pathway and of ERK1/2 in the ERK MAPK pathway, and regulation of CK18 by Wnt is involved in Akt activation. Finally, we discuss the importance of gaining a more complete understanding of the expression of CK18 during carcinogenesis, and suggest potential clinical applications of that understanding. *Mol Cancer Res; 10(4); 485–93. ©2012 AACR.*

Introduction

The intermediate filaments consist of a large number of nuclear and cytoplasmic proteins that are expressed in a tissue- and differentiation-dependent manner. The components of the intermediate filaments are highly conserved during evolution and show a high degree of conservation among species (1, 2). Cytokeratins are major structural proteins found in epithelial cells, which form the cytoplasmic network of intermediate filaments (3). Cytokeratins consist of at least 20 unique gene products that fall into two categories: the relatively acidic type I group (CK9–CK20) and the neutral–basic type II group (CK1–CK8). These cytokeratins are combined in a 1:1 ratio into noncovalent heteropolymers, which are further assembled into keratin filaments (4, 5). The expression of cytokeratin proteins depends primarily on the epithelial cell type and its degree of differentiation; therefore, assessments of cytokeratin expression status are useful for distinguishing carcinomas from other types of cancer (6).

The cytokeratin 18 gene (*CK18*) is located on chromosome 12q13 and is 3,791 base pairs long. It codes for a type I intermediate filament protein that is found primarily in many types of single-layered or "simple" epithelial tissues and is localized in the cytoplasm and perinuclear region (4). CK18 and its coexpressed complementary type II keratin partner, CK8, are persistently expressed in a variety of adult

epithelial organs, such as the liver, lung, kidney, pancreas, gastrointestinal tract, and mammary gland, and are also expressed by cancers that arise from these tissues (7). In the absence of CK8, the CK18 protein is degraded and keratin intermediate filaments are not formed (8). A cDNA clone for CK8 was isolated and expressed in mouse fibroblasts that had previously been transfected with the CK18 gene, which resulted in the formation of stable keratin filaments (9). Thus, the presence of a type I (CK18) and a type II (CK8) keratin appears to be both necessary and sufficient for the formation of keratin filaments (8, 9).

In the first part of this review, we describe the biological functions of CK18 and focus on its roles in cell signaling and in conjunction with various CK18-related proteins. In the second part, we discuss current knowledge about the expression of CK18 in cancers and the potential clinical applications of this knowledge.

Biological Functions of CK18

The known function of CK18 is to provide a flexible intracellular scaffolding to structure cytoplasm, resist stresses externally applied to the cell (1, 2), and maintain normal mitochondrial structures (10). CK18 is also important for cellular processes such as apoptosis (11, 12), mitosis (13), cell cycle progression (14), and cell signaling (15). Furthermore, CK18 has been recognized for many years as an epithelial marker in diagnostic histopathology, and has an important function in tumor cell behavior (Fig. 1; ref. 15).

The dynamic equilibrium of the phosphoglycoprotein CK18 in the soluble and filament pool is an important determinant of its cellular functions, and it is known to be regulated by site-specific phosphorylation (16, 17). Recently, Srikanth and colleagues (18) compared immortalized (Chang) and transformed hepatocyte (HepG2) cell lines and showed that *O*-GlcN-acylation determines the solubility, filament organization, and stability of CK8/18. Studies with several CK18 protein-mimic peptides have shown that CK18

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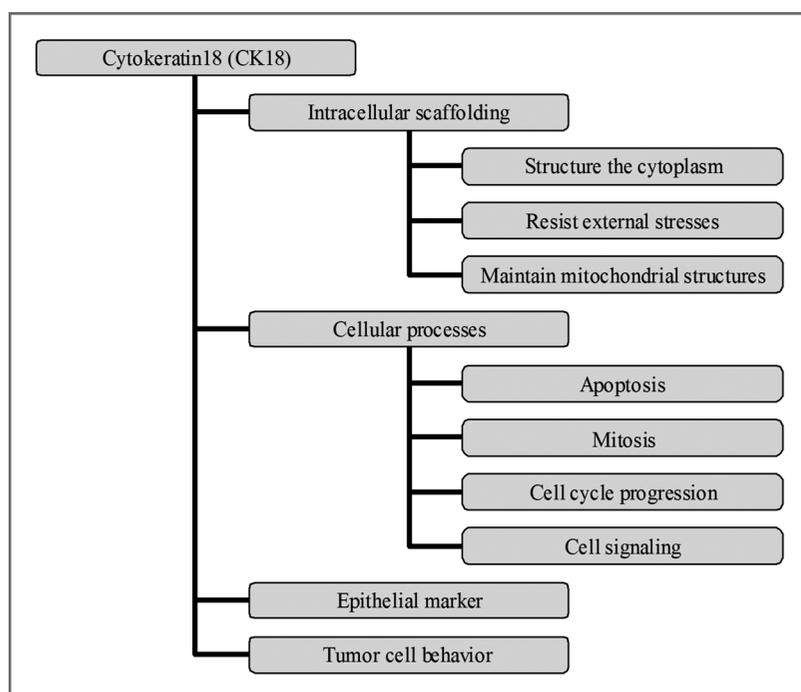
Figure 1. Biological functions of CK18. The functions of CK18 include structuring the cytoplasm, resisting external stresses, and maintaining mitochondrial structures (1, 2, 10). CK18 plays a role in many cellular processes (11–15). It has been recognized as an epithelial marker in diagnostic histopathology and has an important function in tumor cell behavior (15).

glycosylation at Ser48 and phosphorylation at the proximal residue, Ser52, regulate each other through negative feedback systems (19). Leech and colleagues (20) reported that cytokeratins are highly acetylated in a certain colon cancer cell line, with five acetylation sites on CK8 and a further one on CK18. They concluded that the acetylation of CK8/18 may be associated with intermediate filament stabilization and could therefore be a candidate mechanism for the appropriate retention or loss of epithelial cells from flat mucosal layers.

Cell signaling

Galarneau and colleagues (14) proposed that CK18 is involved in the signaling pathways that regulate cell growth, death, and motility. The phosphoinositide 3-kinase (PI3K)/Akt pathway plays a pivotal role in these mechanisms. Studies that evaluated Fas-mediated apoptosis of intestinal epithelial cells showed that the inhibition of PI3K sensitizes cells to Fas-induced apoptosis, whereas the constitutive expression of Akt protects cells from apoptosis (21). CK8/18-free hepatocytes from CK8-null mice were shown to be more sensitive than wild-type mouse hepatocytes to Fas-mediated apoptosis after stimulation with Jo2, an agonistic antibody of Fas ligand (12). CK18 undergoes dynamic O-linked N-acetylglucosamine glycosylation at Ser30, Ser31, and Ser49. Ku and colleagues (22) generated mice that overexpress human CK18 S30/31/49A substitution mutants that cannot be glycosylated (CK18-Gly(−)). Compared with wild-type and other keratin-mutant mice, CK18-Gly(−) mice are more susceptible to apoptosis induced by streptozotocin or to liver injury by combined N-acetyl-D-glucosaminidase inhibition and Fas administration (22). The enhanced apoptosis in the livers of mice that express CK18-Gly(−) involves the inactivation of Akt1 and protein

kinase Cθ as a result of their site-specific hypophosphorylation (22). Akt1 binds to CK8, which probably contributes to the reciprocal hyperglycosylation and hypophosphorylation of Akt1 that occurs upon CK18 hypoglycosylation, and leads to decreased Akt1 kinase activity (22). Therefore, CK18 modified by the addition of an O-linked N-acetylglucosamine residue is shown to be a critical effector of stress-responsive Akt signaling, providing an important link between keratin glycosylation and cell survival (23).

Fortier and colleagues (24) investigated the role of Akt in the regulation of intermediate filament expression in different epithelial cancer cell lines. They found that the overexpression of Akt1 increases the expression of CK8/18 proteins, and Akt2 upregulates CK18 and vimentin expression by increasing mRNA stability. These results indicate that Akt isoforms regulate CK18 expression, and support the hypothesis that CK18 acts as a target of Akt in the PI3K/Akt pathway. However, Galarneau and colleagues (14) detected a stronger degree of Akt activation in CK8-null hepatocytes than in wild-type cells. They also found that insulin stimulation led to a differential Akt activation, implying altered Akt signaling capacity as a result of the CK8/18 loss (14). These results suggest the need for further studies to investigate the role of CK18 in regulating Akt activation and to clarify the feedback loop.

Because the wingless (Wnt) pathway plays a central role in the development of many tissues and organisms, its abnormal activation plays a role in the progression of several major human cancers. In contrast to other secreted Wnt antagonists, Wnt inhibitory factor 1 (WIF1) has been consistently shown to inhibit the growth of various cancer cells both *in vitro* and *in vivo* (25, 26). The levels of phosphorylated Akt were lower in WIF1-overexpressing prostate cancer cells

than in native or control vector-transfected cells. This suggests that WIF1 downregulates the Akt pathway, and that Wnt signaling is involved in Akt activation in prostate cancer cells (26). WIF1 silencing by hypermethylation and consequent Wnt signaling activation has been shown in numerous cancers, including prostate cancer (27). It was reported that restoration of WIF1 expression in prostate cancer cells resulted in decreased cell motility and invasiveness via upregulation of the epithelial markers E-cadherin, CK8, and CK18 (27). Moreover, WIF1 expression significantly reduced tumor growth in a xenograft mouse model, accompanied by increased expression of E-cadherin and CK18 (27). These results show that downregulation of Wnt signaling is accompanied by Akt inactivation and CK18 upregulation in prostate cancer, indicating a role for CK18 in the Wnt pathway.

In a study with adult monkeys, Zhang and colleagues (28) showed that local testicular heat treatment was able to activate the reexpression of CK18 in Sertoli cells, which was coincident with activation of extracellular signal-regulated kinase (ERK)1/2 and Akt kinases. After blocking the ERK mitogen-activated protein kinase (MAPK) signaling pathway, they observed an inhibition of CK18 expression, and they also detected this inhibitory effect by blocking protein kinase A (PKA) activation. However, CK18 activation remained unaltered when the PI3K/Akt pathway was blocked. Zhang and colleagues (28) concluded that the heat treatment was able to induce a reversible change in the Sertoli cells from an adult differentiated state to an immature-like dedifferentiated state through PKA-ERK MAPK-dependent pathways, but not via the PI3K/Akt pathway. In another study, Gilbert and colleagues (29) showed that inhibition of ERK1/2 activation sensitized wild-type but not K8-null mouse hepatocytes to apoptosis, and a much weaker ERK1/2 activation occurred in CK8-null hepatocytes. In turn, this impaired ERK1/2 activation in CK8-null hepatocytes was associated with a drastic reduction in c-Flip

protein, an event that also occurs in a CK8-null mouse mammary cell line. This finding points to a regulatory role of CK8/18 in the c-Flip/ERK1/2 antiapoptotic signaling pathway (29). These two studies indicate that CK18 acts as a target of ERK1/2 and may function as a regulator in the ERK MAPK signaling pathway.

CK18 may affect carcinogenesis through several signaling pathways. Our focus in this review is the role of CK18 in the PI3K/Akt, Wnt, and ERK MAPK signaling pathways. CK18 acts as an identical target of Akt in the PI3K/Akt pathway and of ERK1/2 in the ERK MAPK pathway, and regulation of CK18 by Wnt is involved in Akt activation. It is uncertain whether CK18 functions as a regulator for these three pathways, and further investigations are required to elucidate CK18's functions, account for the existence of the feedback loop, and resolve conflicts in the literature concerning the precise details of the signaling pathways. A hypothetical role for CK18 in cellular signaling during carcinogenesis is illustrated in Fig. 2. CK18, the identical target of the above three pathways, may retroact to Akt and ERK1/2, and the feedback action depends on the functional regulation of CK18. Post-translational modifications such as site-specific phosphorylation and glycosylation are involved in the functional regulation of CK18.

Interactions with related proteins

There is increasing evidence that the functions of CK18 are mediated by interactions with a variety of structural and nonstructural proteins (Tables 1 and 2).

Structural proteins. Plectin is a versatile cytoplasmic cross-linking protein that connects intermediate filaments to microfilaments, microtubules, and membrane adhesion sites. The cross-linking functions of plectin help organize the cytoskeleton into a stable meshwork that is important for maintaining uniformity of cell size and shape. In one study (30), expression of CK18 was shown to be downregulated by suppression of plectin protein, and cytokeratin networks

Figure 2. Hypothetical mechanism for the role of CK18 in carcinogenesis. CK18 acts as an identical target of Akt in the PI3K/Akt pathway (12, 14, 22–24) and of ERK1/2 in the ERK MAPK pathway (28, 29), and regulation of CK18 by Wnt is involved in Akt activation (26, 27). Questions regarding the PI3K/Akt and Wnt pathways, and whether CK18 functions as the regulator in these three pathways require further investigation. CK18* denotes CK18 hypoglycosylation, Akt* denotes hyperglycosylation and hypophosphorylation of Akt1, A solid line ending in an arrowhead (▶) denotes upregulation or activation; a solid line ending in a diamond (♦) denotes downregulation or inhibition; a plain solid line (Đ) denotes uncertain activation or inhibition.
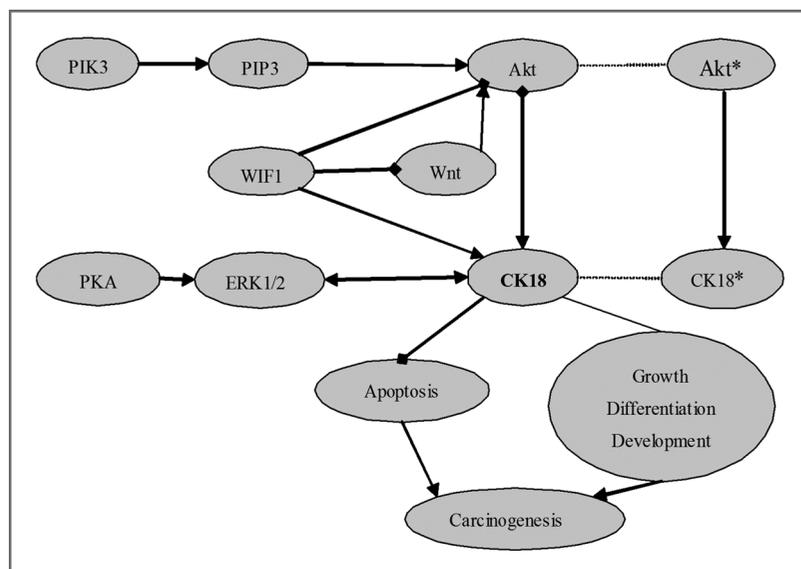
Table 1. Notable features of CK18-related structural proteins

Protein name	Notable features	References
Plectin	Modulates the organization of CK18 and organizes the cytoskeleton into a stable meshwork	(14, 30)
Histone 3	Related to modulation of CK18 and the stability of nucleoskeleton	(31)
Trichoplein	Involved in organization of the apical network of keratin filaments and desmosomes	(32)
Albatross	Regulates the apical junctional complex and lateral domains	(33)
Mrj (molecular chaperones)	Recruits Hsp/c70 to K8/18 and regulates keratin 8/18 filament organization	(34)

were partially collapsed. CK8/18, along with plectin/receptor for activated C kinase 1 (RACK1), has been implicated in the modulation of cell attachment and spreading, size, protein synthesis, and the G_1–S transition (14). Ho and colleagues (31) reported that the H3 histone protein was immunoprecipitated along with CK18 in hepatocellular carcinoma. Their results suggest that expression of the H3 histone is highly correlated with the modulation of CK18, and that an unstable nucleoskeleton may cause instability and fragility of the nucleus.

In addition to plectin and H3 histones, trichoplein and Albatross were also identified as CK8/18-binding proteins (32, 33). Trichoplein shows a low degree of sequence similarity to trichohyalin, plectin, and myosin heavy chain, and colocalizes with CK8/18 filaments in HeLa cells (32). Trichoplein may be involved in the organization of the apical network of keratin filaments and desmosomes in simple epithelial cells (32). Albatross (also known as Fas-binding factor-1) also has a trichohyalin and plectin homology (TPHD) domain, which binds to CK18 directly in a yeast two-hybrid system. Albatross regulates the apical junctional complex and lateral domains in epithelial cells (33). In turn, keratins stabilize Albatross, which promotes the formation of the apical junctional complex. This process may play a critical role in the polarization of epithelial cells. Lastly, a member of the DnaJ/heat shock protein (Hsp) 40 family,

Mrj, binds directly to CK18 and may play an important role in regulating CK8/18 filament organization as a CK18-specific cochaperone with Hsp/c70 (34).

Nonstructural proteins. Several members of the 14-3-3 protein family associate reversibly with CK8/18 in cultured cells and human colonic biopsies but are dependent on both the cell cycle and phosphorylation (35). The association of 14-3-3 proteins with phosphorylated CK8/18 occurs in the cytosolic and membrane-associated compartments during the S and G_2–M phases of the cell cycle (35). Because CK18 Ser33 Ala/Asp mutants inhibit the binding of 14-3-3 proteins and alter keratin organization and distribution, the regulation of keratin/14-3-3 binding during mitosis has been proposed to occur at the CK18 phospho-Ser33 site (16). Phosphorylation of CK8/18 is regulated by a variety of protein kinases, including protein kinase C (PKC) and MAPK (36, 37). Recently, Bordeleau and colleagues (38) identified PKC δ as a mediator of hepatoma cell adhesion and migration, a process that is modulated by CK8/18. They also revealed a CK8/18-dependent relationship between PKC δ and focal adhesion kinase (FAK) activation through an integrin/FAK-positive feedback loop, which was shown to correlate with a reduced FAK residency at focal adhesions (38). In addition, PKC ζ phosphorylates CK18pSer33, which is required for structural reorganization because the KIF network in A549 cells that have been transfected with a

Table 2. Notable features of CK18-related nonstructural proteins

Protein name	Notable features	References
14-3-3	Binds to K18 phosphorylated at Ser33 to modulate keratin organization and distribution	(16, 35)
PKC and MAPK	Regulates phosphorylation of CK8/18	(36–39)
p38 and MK2	Cooperatively phosphorylate epithelial keratins	(17)
TNFR2	Moderates TNF-induced JNK intracellular signaling and NFκB activation	(11)
TRADD	Attenuates TNF-induced apoptosis	(40)
DEDD	Regulates degradation of CK8/18 filaments in apoptosis	(41, 42)
Pirh2	Regulates keratin filament organization to influence mitochondria distribution	(43, 44)
LRP16	CK18 associates with and sequesters LRP16 in regulating the ERα signaling pathway	(45, 46)
HPV16 E1 = E4	Acts as a keratin cross-linker and prevents the movement of keratins between the soluble and insoluble compartments	(47)
Chymotrypsin C	Regulates pancreatic cancer cell migration in relation to CK18 expression	(48)

dominant negative PKC ζ or express the CK18Ser33Ala mutation is unchanged (39). The MAPK-activated protein kinases MK2 and MK3 are directly activated via p38 MAPK phosphorylation, and their function is to contribute to the stress response and stabilize p38 through the formation of complexes. The phosphorylation of CK8-Ser73 is catalyzed directly by p38, the expression of which is in turn dependent on MK2 (17). Because CK8 is the coexpressed complementary type II keratin partner of CK18, the latter theoretically can also be phosphorylated by p38. Of interest, *in vitro* studies have shown that MK2, but not p38, directly phosphorylates CK18-Ser52 (17).

TNF acts through two distinct receptors [TNF receptor 1 (TNFR1) and TNFR2] and influences cellular proliferation, differentiation, survival, and death. CK8 and CK18 both bind to the cytoplasmic domain of TNFR2 and moderate TNF-induced c-*jun*-NH$_2$-kinase (JNK) intracellular signaling and NFκB activation (11). CK18 is also known to bind specifically to the TNFR1-associated death domain protein (TRADD) through its N-terminal region. Once bound to CK18, TRADD cannot bind to the activated TNFR1, thus attenuating TNF-induced apoptosis in simple epithelial cells (40).

Early in apoptosis, the cytosolic death effector domain-containing DNA-binding protein (DEDD) and its close homolog, DEDD2, form filaments that associate with and are dependent on CK8/18 and active caspase-3. siRNA-mediated DEDD knockdown cells exhibit inhibition of staurosporine-induced DNA degradation. This suggests that DEDD regulates the degradation of CK8/18 filaments during apoptosis (41). In similarity to this finding, it was shown that CK8/18 loss induces a switch in Fas-induced death signaling, likely through the involvement of a DEDD (42).

The CK8/18-interacting partner, Pirh2, was identified as a RING-H2–type ubiquitin E3 ligase that targets p53 degradation (43, 44). Binding of Pirh2 to CK8/18 regulates keratin filament organization, which further influences the cellular distribution of mitochondria. A disruption in the interaction between Pirh2 and CK8/18 leads to an altered cellular distribution of mitochondria and enhanced UV-induced apoptosis (44).

The estrogen receptor α (ERα) target gene and coactivator, LRP16, plays a crucial role in ERα activation and the proliferation of MCF-7 breast cancer cells (45). CK18 can effectively associate with and isolate LRP16 in the cytoplasm, thereby attenuating the final output of ERα-mediated signaling and the progression of the estrogen-stimulated cell cycle. Loss of CK18 expression increases the functional availability of LRP16 to ERα and promotes the proliferation of ERα-positive breast tumor cells. These findings indicate that CK18 plays an important functional role in regulating the ERα pathway (46). High-risk human papillomaviruses, such as human papillomavirus type 16 (HPV16), are the primary cause of cervical cancer. The HPV16 E1 = E4 protein binds to keratins directly and interacts strongly with CK18. By associating with keratins through its N-terminus, and with itself through its C-terminus, 16E1 = E4 may act as

a keratin cross-linker and prevent the movement of keratins between the soluble and insoluble compartments (47). Another CK18-related protein is chymotrypsin C. Wang and colleagues (48) reported that the amount of chymotrypsin C in pancreatic cancer cells is only 20% of that found in normal cells, and that among 26 identified differential proteins, CK18 was the most obviously correlated with chymotrypsin C expression. The authors speculated that chymotrypsin C may regulate pancreatic cancer cell migration in relation to CK18 expression.

In summary, CK18 performs its biological functions and plays a role in carcinogenesis in conjunction with various known and unknown related proteins. The CK18-related proteins not only help organize and modulate cytokeratin networks but also serve important functions in various cellular processes and tumor cell behavior by interacting with CK18. Further investigations are needed to study the association between CK18 and CK18-related proteins.

Expression and Clinical Applications of CK18 in Cancer

Expression of CK18

CK18 has been recognized for $\sim$30 years as a structural marker protein that is specific to epithelial cells and consequently is involved in both cell motility and cancer progression (49). The expression of CK18 is associated with patient prognosis in a variety of cancers (50, 51).

Makino and colleagues (49) reported that increased CK18 expression was correlated with poor differentiation and advanced stage in esophageal squamous cell carcinoma. Patients with CK18-positive tumors had a poorer prognosis compared with patients with negative esophageal squamous cell carcinoma (49). In renal cell carcinoma, CK18 mRNA and protein levels increased with advanced stage and grade (52). A similar result was also obtained in other malignant diseases, such as oral cavity carcinoma (50) and lung cancer (53). In contrast, some CK18-expressing adenocarcinomas show decreased expression with increasing tumor progression, which correlates with a poor outcome in human breast (51, 54) and colorectal cancer (55). Substantial CK18 downregulation was observed in nasopharyngeal carcinoma compared with normal nasopharyngeal epithelial tissues (56). The downregulation of CK18 was significantly correlated with poor histological differentiation, whereas CK18 upregulation was significantly correlated with advanced clinical stage, recurrence, regional lymph node metastasis, distant metastasis, and poor prognosis. *In vitro* analyses using a CK8/18 transfection technique also yielded conflicting results: In one study, mouse L cells transfected with CK8 and CK18 showed enhanced migration and invasive ability (57), whereas in another study, transfection of the CK18 gene into human breast cancer cells caused a marked regression of their malignant potential (58). These conflicting results for the expression of CK18 fragments in carcinomas and the prediction of patient prognosis may result from differences among the types of carcinoma studied, or among the experimental protocols used by the different research groups.

The dysfunctional regulation of CK18 may play a vital role in the pathogenesis of different cancers. Further investigation is needed to determine the consequences of altered expression of CK18 on cellular function. Investigators have explored various post-translational modifications of CK18, including site-specific phosphorylation (16, 19, 21), O-GlcN-arylation (18, 19), and acetylation (20). These modifications regulate each other and determine the solubility, filament organization, and stability of CK8/18. This may significantly alter the role of CK18 in carcinogenesis. It is therefore plausible that CK18 may play profound roles in cancer development, invasion, and metastasis, and any further evidence about the regulatory and pathogenic mechanisms of different carcinomas would be of great importance.

Clinical applications of CK18

The expression of CK18 may serve as a differential diagnostic marker in various cancers. Nagashio and colleagues (53) reported that CK18 was upregulated in large cell neuroendocrine carcinoma compared with small cell lung carcinoma. Another study also showed substantially higher levels of positive CK18 immunostaining in adenocarcinoma and large cell neuroendocrine carcinoma than in squamous cell carcinoma and small cell lung carcinoma (59). In breast cancer, reduced CK8/18 expression in conjunction with the basal-like phenotype and family history may improve investigators' ability to identify which tumors are likely to be associated with a BRCA1 germline mutation, and thus may help streamline genetic testing (60). In addition, CK18 expression has been used to distinguish the sites of origin of various head and neck squamous cell carcinomas (61).

Both full-length and caspase-cleaved CK18 fragments can be detected in the peripheral circulation of patients with carcinomas, and are believed to reflect cell death of epithelial origin. Two sandwiched ELISA assays, M30 and M65, can be used to determine different circulating forms of CK18 in either plasma or serum, and have been proposed to be surrogate biomarkers of different mechanisms of cell death (62, 63). The M30 ELISA assay uses the M5 antibody as a catcher and the M30 antibody to detect caspase-cleaved CK18 produced during the early stages of apoptosis (62). The M65 assay also detects cleaved fragments but uses a different detection antibody from M30, which does not distinguish between the full-length protein and its fragments. The M65 assay theoretically measures both caspase cleavage and cellular release of intact CK18 (63).

Serum M65 and M30 levels were shown to be elevated in patients with different types of carcinoma (64–68). The measurement of caspase-cleaved or total CK18 from epithelial-derived tumors could be a simple, noninvasive way to monitor or predict tumor progression (69), prognosis (64, 65, 69, 70), and response to chemotherapy (66–68, 70–72).

In a study by Koelink and colleagues (69), plasma caspase-cleaved and total CK18 levels in colorectal cancer patients were related to disease stage and tumor diameter. The caspase-cleaved/CK18 ratio, which decreased with tumor progression, was also predictive of disease-free survival, with a low ratio associated with worse disease-free survival. In a study of patients with advanced gastric carcinoma, the patient population with higher M30 levels had significantly shorter median survival rates than the population with lower serum M30 levels, whereas there was no impact of serum M65 levels on survival (64). This was in contrast to the results of Bilici and colleagues (73), who showed that increased plasma levels of both M30 and M65 could predict progression-free survival in patients with gastric cancer. This discrepancy was also observed in studies of lung cancers. In one study, the patient population with higher basal M30 antigen levels had significantly shorter median survival rates than the group with lower levels (70). In another study, total CK18 showed potential as a prognostic marker in patients with non–small cell lung cancer, whereas caspase-cleaved CK18 had no prognostic value (65). Different investigators have reported encouraging data regarding the potential use of CK18 as a serum biomarker to assess the treatment efficacy of chemotherapy for various carcinomas, including gastrointestinal carcinomas (66, 67), breast cancer (71, 72), and lung cancer (70).

However, the situation in vivo may be more complicated. For example, baseline plasma CK18 levels in pancreatic cancer are affected by the presence of obstructive jaundice and prolonged plasma storage (74). Clinical biomarker studies that use serial CK18 levels are warranted in cancer, given the presence of potentially confounding factors. In general, the significance of detecting circulating caspase-cleaved and total CK18 requires further evaluation in larger patient groups.

Conclusions

CK18 and its complementary partner, CK8, are the most common and characteristic members of the large intermediate-filament gene family that are expressed in "simple" or single-layer epithelial tissues of the body (4). CK18 may modulate intracellular signaling and apoptosis via interactions with various related proteins. There is evidence to suggest that CK18 is involved in the invasive or growth properties of tumors (49, 50). However, conflicting results have been obtained in vitro (58) and in vivo (51, 54, 55). Although these discrepancies may be due in part to differences among the carcinoma types studied or the experimental protocols used by different research groups, the precise roles of CK18 are currently unknown.

Here, we propose a hypothetical mechanism for the function of CK18 in carcinogenesis. CK18 acts as an identical target in the PI3K/Akt and ERK MAPK pathways, and is regulated by Wnt via Akt activation (Fig. 2). However, it is uncertain whether CK18 may function as a regulator for these three pathways, and it is also unclear what the function of the feedback action of CK18 is under physiological conditions. Further investigations will be necessary to explore the regulatory mechanisms of CK8/18 expression, as well as their exact functions in cell signaling. Studies on the

consequences of post-translational modifications of CK18, including site-specific phosphorylation, *O*-GlcN-arylation, and acetylation, are required. The identification of novel CK18-related proteins and further studies in transgenic mouse models and human diseases would provide evidence of the precise functions of CK18. Large clinical studies would allow investigators to evaluate the relationship between caspase-cleaved CK18 fragments and disease risk in patients with cancer, as well as therapeutic responses. CK18 shows considerable promise as an important marker of therapeutic efficacy (66, 67, 70–72). Further, in combination with other markers, the use of CK18 could be an important step toward preselecting patients to determine the most appropriate therapy for their particular disease status.

Disclosure of Potential Conflicts of Interest

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