Metastasis is one of hallmarks of cancer and a major cause of cancer death. Combatting metastasis is highly challenging. To overcome these difficulties, researchers have focused on physical properties of metastatic cancer cells. Metastatic cancer cells from patients are softer than benign cancer or normal cells. Changes of viscoelasticity of cancer cells are related to the keratin network. Unexpectedly, keratin network is dynamic and regulation of keratin network is important to the metastasis of cancer. Keratin is composed of heteropolymer of type I and II. Keratin connects from the plasma membrane to nucleus. Several proteins including kinases, and protein phosphatases bind to keratin intermediate filaments. Several endogenous compounds or toxic compounds induce phosphorylation and reorganization of keratin network in cancer cells, leading to increased migration. Continuous phosphorylation of keratin results in loss of keratin, which is one of the features of epithelial mesenchymal transition (EMT). Therefore, several proteins involved in phosphorylation and reorganization of keratin also have a role in EMT. It is likely that compounds controlling phosphorylation and reorganization of keratin are potential candidates for combating EMT and metastasis.

Key Words: Metastasis, Viscoelasticity, Phosphorylation of keratin, Reorganization of keratin, Epithelial Mesenchymal Transition, Sphingosylphosphorylcholine
STRUCTURE AND CHARACTERISTICS OF KERATINS

Epithelial cell keratins are composed of heteropolymer of one type I keratin and one type II keratin proteins (Table 1) (Coulombe and Omary, 2002). Keratin contains a common α-helical rod domain of ~310 amino acid, sided by non-helical head and tail domains of diverse length and sequence having several phosphorylation sites (Ku et al., 1998; Omary et al., 2006; Loschke et al., 2015) (Fig. 1).

Table 1. Expression of keratin proteins in epithelial tissues*

| Keratin | Epithelial tissue | Partner |
|---------|-------------------|---------|
| Type I  |                   |         |
| Simple  |                   |         |
| K18     | Simple epithelia (e.g. liver, pancreas, colon, lung) | K8, K7 |
| K20     | Simple epithia, especially gastrointestinal | K8, (K7) |
| Barrier |                   |         |
| K9      | Stratified cornifying epithelia; palm, sole | (K1) |
| K10     | Stratified cornifying epithelia; suprabasal | K1 |
| K12     | Stratified epithelia; cornea | K3 |
| K13     | Stratified epithelia; non-cornifying; suprabasal | K4 |
| K14     | Stratified and complex epithelia; basal | K5 |
| K15     | Stratified epithelia | (K5) |
| K16     | Stratified epithelia; induced during stress, fast turn over; suprabasal | K6a |
| K17     | Stratified epithelia; induced during stress, fast turn over | K6b |
| K19     | Simple and stratified epithelia | K8 |
| K23, K24| Epithelia | |
| Structural |                 |         |
| K25, K26, K27, K28 | Stratified epithelia; hair follicle sheath | |
| K31, K32, K33a, K33b, K34, K35, K36, K37, K38, K39, K40 | Stratified epithelia; hair, hard structure | |
| Type II |                   |         |
| Simple  |                   |         |
| K7, K8  | Simple epithia | K18 |
| Barrier |                   |         |
| K1      | Stratified cornifying epithelia; suprabasal | K10 |
| K2      | Stratified cornifying epithelia; late suprabasal | (K10) |
| K3      | Stratified epithelia; cornea | K12 |
| K4      | Stratified epithelia; non-cornifying; suprabasal | K13 |
| K5      | Stratified and complex epithelia; basal cells | K14, (K15) |
| K6a     | Stratified epithelia; induced during stress, fast turn over | K16 |
| K6b     | Stratified epithelia; induced during stress, fast turn over | K17 |
| K6c     | Epithelia | |
| K76     | Stratified cornifying epithelia, oral, suprabasal | (K10) |
| K78, K79, K80 | Epithelia | |
| Structural |                 |         |
| K75     | Stratified epithelia; hair follicle | |
| K71, K72, K73, K74 | Stratified epithelia; hair follicle sheath | |
| K81, K82, K83, K84, K85, K86 | Stratified epithelia; hair, hard structure | |

*Modified from Haines and Lanes, and Loschke (Haines and Lane, 2012; Loschke et al., 2015).

Epithelial cell keratins are composed of heteropolymer of one type I keratin and one type II keratin proteins (Table 1) (Coulombe and Omary, 2002). Keratin contains a common α-helical rod domain of ~310 amino acid, sided by non-helical head and tail domains of diverse length and sequence having several phosphorylation sites (Ku et al., 1998; Omary et al., 2006; Loschke et al., 2015) (Fig. 1).

Simple epithelia of liver, intestine, and pancreas, are discovered as pairs of K7, K8, K18, K19, and K20, but the ratio of type I and type II keratins is 1:1 in all cells (Moll et al., 1982; Ku et al., 1999; Toivola et al., 2002). K8 and K18 assemble to form heterodimers in epithelia of gland (Omary et al., 2009; Toivola et al., 2015). Keratins assemble as heterodimers of each of type I and type II keratin monomer, aligned in parallel (Hatzfeld and Weber, 1990; Herrmann and Aebi, 2000; Haines and Lane, 2012). These heterodimers convert to antiparallel tetramers by overlaying the N-terminal half of rod domains and tetramers then form ‘unit length filaments’ (60 nm in length) (Fig. 2) (Haines and Lane, 2012).

Several situations including diverse stress requires the changes of keratins (Leube et al., 2011). Keratin cycle starts with nucleation of keratin units at the peripheral region of cells including vicinity of focal adhesions (Windoffer et al., 2011). Next, elongation of new keratin units follows actin-dependent movement toward the peripheral keratin network (Windoffer et al., 2011). After consolidation of keratin particles to keratin network, keratin filaments keep to move toward the rim of nucleus and bundle (Loschke et al., 2015). Parts of keratins break up into several pieces of oligomers that diffuse into the cytosol (Loschke et al., 2015). Other keratins make a peri-
nuclear keratin network and are linked to desmosome and hemidesmosome (Fig. 3) (Windoffer et al., 2011).

**KERATIN IN THE EPITHELIAL CELLS**

In the epithelia tissues, a network of proteins links the nu-
Keratin in epithelial cells. Desmosome junction: Desmosomes link to the keratin filament of cells. Transmembrane desmosomal cadherins, desmoglein and desmocollin, bind placoglobin, the armadillo family protein, which holds the plectin, plakin family member (Fuchs and Raghavan, 2002). The cytoplasmic plaque anchors the keratin intermediate to the desmosome. Hemidesmosome junction: Integrin α and β heterodimers consist of the core of the hemidesmosome, along with BPAG2, a transmembrane protein. BPAG1e and plectin are two hemidesmosomal proteins that are members of the plakin family (Haines and Lane, 2012). They seem to function by connecting the keratin filament to the transmembrane proteins in the hemidesmosome. BPAG1e, bullous pemphigoid antigen 1, epidermal isosulfatase; BPAG2, bullous pemphigoid antigen 2 (Haines and Lane, 2012). Nuclear junction: Nesprin 3 attach to SUN proteins through the perinuclear space and can directly connect to keratin proteins via plectin (Gerlitz and Bustin, 2011). Modified and combined from Fuchs and Raghavan, Gerlitz and Bustin, and Haines and Lanel (Fuchs and Raghavan, 2002; Gerlitz and Bustin, 2011; Haines and Lane, 2012).

Linking to nucleoplasm and hemidesmosome

Keratin is connected to desmosome in the cell to cell adhesion site through desmoplakin (Green and Simpson, 2007). The cadherin family, the desmogleins and desmocollins, join the adhesion point (Getts et al., 2004; Green and Simpson, 2007). The tails of the cadherins give an association region for the armadillo proteins such as plakoglobin, plakophilins 1-3, and p0071 (Schmidt and Jager, 2005; Green and Simpson, 2007). The carboxy terminal of desmoplakin interacts directly with the amino terminal end of type II keratins (Fig. 4) (Kouklis et al., 1994; Hatsell and Cowin, 2001).

Hemidesmosomes are junction complexes contributing to the adherence of epithelial cells to the basal layer (Borradori and Sonnenberg, 1999). The molecular structure of hemidesmosome is composed of 3 kinds of proteins: the cytoplasmic linker proteins for intermediate filaments at the cytoplasmic leaflet of the plasma membrane, the transmembrane proteins acting as receptors linking the inside of cell to the proteins of the basal layers (Borradori and Sonnenberg, 1999). Keratin is linked to plectin and BPAG1e at hemidesmosome cell-matrix adhesions (Guo et al., 1995; Green and Simpson, 2007; Pan et al., 2013). The linking of plectin to keratins is required for hemidesmosome assembly (Fig. 4) (Koster et al., 2004). Keratins localize hemidesmosomes and repress migration of cells (Seltmann et al., 2013).

Linking to nucleoplasm

Nesprins are a core member of the LINC (Linker of Nucleoskeleton and Cytoskeleton) complex that cross over both nuclear membranes to link the cytoplasm and the inside of nucleus (Neumann and Noegel, 2014). Nesprins interact with SUN proteins through perinuclear space via their KASH (Klarsicht, ANC-1, Synex Homology) domain and directly link to actin filaments (nesprin-1 and -2) and keratins via plectin (nesprin-3) (Padmakumar et al., 2005; Friedl et al., 2011). The cytoplasmic N-terminus of nesprin-3 interacts with plectin, a member of the plakin family of cytoskeletal linker protein (Sonnenberg and Liem, 2007). Nesprin-1 and -2 bind to microtubules via kinesin or dynein...
Linking to microfilaments & microtubules
Keratin particles emerge from the vicinity of the plasma membrane, maneuver continuously toward the central part of cell, and consolidate into the peripheral keratin network (Kolsch et al., 2009). These keratin cycles are highly dependent on interaction with actin filament (Pan et al., 2013). Actin depolymerization rapidly triggers keratin intermediate filament formation by turning on keratin related genes (Chang et al., 2014).

Keratin particles also moves fast via microtubules (Liovic et al., 2003). Keratin shows 2 types of motility in cells such as slow, continuous transport of keratin precursor particles of cell, and fast, bidirectional movement of keratin particles (Woll et al., 2005). Type I movement is mediated by actin and type II movement is mediated by microtubule systems (Woll et al., 2005).

Spectroplakins are big cytoskeletal linking proteins that bind to all 3 members of the cytoskeleton such as actin filaments, microtubules, and intermediate filaments (Suozzi et al., 2012). The spectroplakin family is composed of two mammalian genes, MACF1 (Microtubule-Actin Crosslinking Factor 1), and Dst (Dystonin) encoding bullous pemphigoid antigen 1 (Suozzi et al., 2012). BPAG1 connects the keratin network to hemidesmosome of cell to intensify the mechanical strength at the basal layer of the epidermis (Koster et al., 2003; Suozzi et al., 2012).

PHOSPHORYLATION OF KERATINS
A wide range of post-translational modifications have been reported on keratins such as phosphorylation, ubiquitylation, acetylation, glycosylation, and, sumoylation, which seem to control the solubility of keratins in several situations (Omary et al., 2006; Ku et al., 2010; Srikanth et al., 2010; Snider et al., 2011). Recently, a review focuses on post-translational modification of intermediate filament proteins including vimentin and keratin (Snider and Omary, 2014). So we just emphasize phosphorylation of keratin which is key event in perinuclear reorganization of keratin (Beil et al., 2003).

Phosphorylation is a key reaction of keratins, and K1, K8, K18, and K19 are the fully studied among keratin family (Steinert, 1988; Zhou et al., 1999; Omary et al., 2002). Multiple factors such as several stresses, apoptosis, and mitosis, regulate keratin phosphorylation resulting keratin filament reorganization (Ku et al., 1999). Serine is the primary amino acid of phosphorylated keratin (Oshima, 1982; Omary et al., 1998). Tyrosine and threonine are also phosphorylated keratin residues (Feng et al., 1999). Sphingosylphosphorylcholine (SPC)-induced phosphorylation and perinuclear reorganization of keratin are implicated in viscoelasticity of PANC-1 cancer cells (Beil et al., 2003). Therefore, keratin phosphorylation seems
to be important in regulating the physical properties of cancer cells. However, it is not yet clear that perinuclear reorganization by phosphorylation is a special event for metastatic cancer or just one step of keratin recycle process. In addition, it is not clear why metastatic cancer cells reveal phenotypes such as the perinuclear reorganized keratin structure.

### PLAYERS INVOLVED IN PHOSPHORYLATION AND REORGANIZATION OF KERATINS

#### Mitogen-activated protein (MAP) kinases

Numerous kinases are involved in phosphorylation of keratins (Snider and Omary, 2014). Phosphorylation of serine residue of keratin leads to disintegration of the stable structure and increased solubility of keratin in the cytoplasm (Omary et al., 1998).

ERK is one of the kinases involved in SPC or leukotriene B4 (LTB4)-evoked K8 phosphorylation and reorganization (Fig. 5, Table 2) (Busch et al., 2012; Park et al., 2012). ERK is also required in acetone extracts from Bupleurum scorzonerifolium-induced K8 phosphorylation in A549 cancer cells (Chen et al., 2005).

Serine-73 (Ser-73) of K8 is a residue of phosphorylation by c-Jun N-terminal kinase (JNK) (Fig. 5, Table 2). Furthermore, we found that JNK phosphorylates serine-431 (Ser-431) in SPC-induced phosphorylation and reorganization of K8 (He et al., 2002; Park et al., 2011).

p38 mitogen activated protein kinase (MAPK) is also involved in phosphorylation of Ser-73 induced by treatment with okadaic acid or orthovanadate (Ku et al., 2002a; Woll et al., 2007). p38 MAPK phosphorylates MAPK-activated protein kinase MK2 and phosphorylation of Ser-73 in HT29 cells is dependent on MK2 (Fig. 5, Table 2) (Menon et al., 2010). MK2 also phosphorylates Ser-52 of K18 and Ser-13 of K20 (Menon et al., 2010).

#### PKA, PKC, and CAMK II

cAMP-dependent protein kinase (PKA) and Ca^{2+}-dependent protein kinase C (PKC) almost exclusively phosphorylates serine of K8 (Fig. 5, Table 2) (Yano et al., 1991). PKA phosphorylates Ser-8, Ser-12, Ser-23, Ser-33, Ser-36, Ser-42, and Ser-50 in the head domain and Ser-416, Ser-423, and Ser-425 in the tail region of K8 (Ando et al., 1996). Protein kinase C (PKC) phosphorylates K8 at Ser-8 and Ser-23 in thyroprotin-releasing hormone (TRH) -treated GH4C1 cells (Akita et al., 2007). Interestingly, PKCε and K8 have perinuclear colocalization under basal conditions and are found in the cell periphery and cell to cell contact region after TRH treatment (Akita et al., 2007). Protein kinase C (PKC) phosphorylates Ser-73 of K8 regulating the shear stress-mediated collapse of keratin network in human A549 cells (Ridge et al., 2005). Protein kinase C (PKC) phosphorylates Ser-33 of K18 leading to reorganization of keratin proteins induced by shear stress (Sivaramakrishnan et al., 2009). Phosphorylation of Ser-13 of K20 is increased after PKC activation but it is not clear whether PKC phosphorylates Ser-13 of K20 (Zhou et al., 2006). Recently, K8 phosphorylation by PKC is known to a major contributing factor for K8 downregulation in human disc degeneration (Sun et al., 2013).

Calmodulin-dependent protein kinase II (CAMK II) phosphorylates K8 at serine and threonine amino acids (Yano et al., 1991). However, specific sites of phosphorylated residues of K8 by CAMK II were not reported.

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### Table 2. Phosphorylated residues of keratins and kinases involved

| Keratins | Phosphorylated residues | Kinases involved | References |
|----------|-------------------------|-----------------|------------|
| K8       | Ser-8                   | PKA, PKC,       | (Akita et al., 2007; Ando et al., 1996) |
|          | Ser-12                  | PKA             | (Ando et al., 1996) |
|          | Ser-23                  | PKA, PKC,       | (Akita et al., 2007; Ando et al., 1996) |
|          | Ser-33                  | PKA             | (Ando et al., 1996) |
|          | Ser-36                  | PKA             | (Ando et al., 1996) |
|          | Ser-42                  | PKA             | (Ando et al., 1996) |
|          | Ser-50                  | PKA             | (Ando et al., 1996) |
|          | Ser-73                  | JNK, PKC, MK2*  | (He et al., 2002; Menon et al., 2010; Ridge et al., 2005) |
|          | Ser-416                 | PKA             | (Ando et al., 1996) |
|          | Ser-423                 | PKA             | (Ando et al., 1996) |
|          | Ser-425                 | PKA             | (Ando et al., 1996) |
|          | Ser-431                 | ERK, JNK        | (Busch et al., 2012; Park et al., 2011; Park et al., 2012) |
|          | Not determined           | AKT, AMPK, CAMK II, CK-IA, | (Kuga et al., 2013; Loschke et al., 2015; Velasco et al., 1998; Yano et al., 1991) |
| K17      | Ser-44                  | RSK1            | (Pan et al., 2011) |
|          | Not determined           | US3             | (Murata et al., 2002) |
| K18      | Ser-33                  | PKC             | (Sivaramakrishnan et al., 2009) |
|          | Ser-52                  | MK2             | (Menon et al., 2010) |
|          | Not determined           | AMPK            | (Velasco et al., 1998) |
| K19      | Ser-35                  | Not determined  | (Zhou et al., 1999) |
|          | Tyr-391                 | Src kinase      | (Zhou et al., 2010) |
| K20      | Ser-13                  | MK2, PKC*       | (Menon et al., 2010; Zhou et al., 2006) |

*No evidence for phosphorylation of residue by indicated kinase but dependent on that.
AKT and RSK

Predicted phosphorylation sites for Akt exist in several keratins and Akt binds K8 but not K18 (Fig. 5, Table 2) (Paramio et al., 2001; Loschke et al., 2015). In the presence of K8 and K18, K8-Akt interaction is independent of K18 glycosylation and Thr 308-phosphorylation in Akt1 (Ku et al., 2010). Akt1 overexpression also increases K8 and K18 proteins (Fortier et al., 2010b). However, there are no reports on Akt-induced phosphorylation of specific residue(s) of keratin.

K17, a type I keratin, is heavily induced in epidermis after injury, and in psoriasis and cancer (Pan et al., 2011). p90 ribosomal protein S6 kinase 1 (RSK1) phosphorylates Ser-44 residue of K17 of keratinocytes (Fig. 5) (Pan et al., 2011). However, this phosphorylation is not clearly linked to a modification of keratin network.

Casein kinase 1α

Casein kinase 1α (CK-1α) plays an essential role in the phosphorylation and degradation of β-catenin (Knippschild et al., 2005). Casein kinase 1α (CK-1α) mediates FAM83H (family with sequence similarity 83 member H)-dependent reorganization of keratin filaments (Kuga et al., 2013). Inhibition of CK-1α is a cause of keratin filament bundling and reverses keratin filament disassembly; but it is not yet known which amino acid residue of K8 or K18 is phosphorylated by CK-1α. Ser-73 and Ser-431 of K8 and Ser-33 and Ser-52 of K18 are not candidates of substrates of CK-1α (Fig. 5, Table 2) (Kuga et al., 2013).

Src kinase

Ser-35 of K19, which is a type I keratin, is a well-known residue of phosphorylation (Zhou et al., 1999). Src kinase phosphorylates tyrosine 391 of human K19 (Fig. 5, Table 2) (Zhou et al., 2010). During keratinocytes migration and tissue repair, Src kinase activity is inhibited by wound-induced keratin such as K6a and K6b (Rotty and Coulombe, 2012).

Miscellaneous kinases

The AMP-activated protein kinase (AMPK) is important in the biological response induced by metabolic changes and is turned on by AMP (Velasco et al., 1998). AMPK and 5-aminoimidazole-4-carboxamide ribonucleotide, a AMPK activator phosphorylate K8 and K18 in primary hepatocytes (Fig. 5, Table 2) (Velasco et al., 1998).

US3 is a specific serine/threonine protein kinase found in herpes simplex virus (Murata et al., 2002; Koyanagi et al., 2014). US3 protein kinase directly phosphorylates K17 (Fig. 5, Table 2) (Murata et al., 2002). However, there are no reports on US3 or AMPK-induced phosphorylation of specific residue(s) of keratin.

OTHER PLAYES IN KERATIN PHOSPHORYLATION AND REORGANIZATION

Protein phosphatase

Several kinases are reportedly implicated in the SPC-induced phosphorylation of K8. For example, ERK and JNK are involved in SPC-induced K8 phosphorylation (Park et al., 2011; Busch et al., 2012). So common upstream regulator of ERK and JNK might be important in SPC-induced K8 phosphorylation. Protein phosphatase-2A (PP2A) dephosphorylates phospho ERK and phospho JNK (Fig. 5) (He et al., 2002; Hu et al., 2009). PP2A directly dephosphorylates K8 during hyposomotic stress in HT29 cells (Tao et al., 2006). PP2A also maintains the structure and interactions of hepatic keratin intermediate filaments (Toivola et al., 1997). PP2A down regulation is also involved in LTB4-evoked phosphorylation of K8 at Ser-431 (Park et al., 2012).

Phosphatase of regenerating liver-3 (PRL-3) belongs to the PRL protein tyrosine phosphatase family and highly PRL-3 expressed cancer cells demonstrate reduction of K8 phosphorylation, especially at the front of invasion and metastasis to liver (Fig. 5) (Mizuuchi et al., 2009). Especially, loss of plakophilin 3 results in an increase in PRL3 levels promoting K8 dephosphorylation of HCT116 cells (Khapare et al., 2012).

Pharmacological inhibition of the protein-tyrosine phosphatase PTP1B increases phosphorylation of Tyr-267 of K8, decreases solubility, and increases K8 filament bundling, whereas PTP1B overexpression has the opposite effects (Fig. 5) (Snider et al., 2013).

It seems that effects on K8 structure and stability by phosphorylation of serine differ from those of tyrosine phosphorylation. Further study is needed to elucidate the role of different phosphorylated keratins on structure and reorganization.

Miscellaneous binding partner of keratins

High-risk human papillomaviruses (HPV) such as HPV16, are the major cause of cervical cancer and one of HPV16 proteins, E1-E4 binds to keratins leading to keratin network disorganization (Fig. 5) (Wang et al., 2004). Albatross exists with keratin filaments in nonpolarized epithelial cells and keratins stabilize the Albatross protein (Fig. 5) (Sugimoto et al., 2008). A newly identified keratin-associated protein, FAM83H regulates the filamentous state of keratins and the C-terminal region of FAM83H interacts with keratins (Fig. 5) (Kuga et al., 2013).

Death effector domain with DNA binding protein (DED), is present mostly as mono- or diubiquitinated form, and diubiquitinated DEDD bind to the K8 and K18 (Fig. 5) (Lee et al., 2002). Migration inhibitory factor-related protein 8 (MRP8) and MRP14, may be implicated in Ca2+-induced keratins reorganization in TR146 human squamous cell carcinoma (Fig. 5) (Goebele et al., 1995). p53-induced ubiquitin-protein ligase (Pirh2), binds to K8 and K18 and phosphorylation of either Pirh2 or K8 and K18, influences their binding (Fig. 5) (Duan et al., 2009).

Association of small heat shock proteins (HSP) with intermediate filament including keratins, may regulate filament interactions in cellular networks. For example, the chaperone HSP27 affects assembly dynamics and organization of K8 and K18 cytoskeleton through direct keratin interactions (Fig. 5) (Perger et al., 1999; Kayser et al., 2013; Loschke et al., 2015).

14-3-3 protein binds to several kinases of signal transduction (Liao and Omary, 1996). 14-3-3 proteins also interact with phosphorylated form of keratin in simple epithelia during the course of cell cycle and plays a role of cofactor for solubilization of keratins (Liao and Omary, 1996). Ser-33 phosphorylation of K18 influences binding of K18 to 14-3-3 proteins in the course of mitosis and interaction of K18 with 14-3-3 proteins regulates keratin filaments and mitotic progression of hepatic cells (Fig. 5) (Ku et al., 2002b).
**INDUCERS OF PHOSPHORYLATION AND REORGANIZATION OF KERATINS**

### Growth factor & cytokines

Epidermal growth factor (EGF) leads to phosphorylation of keratin in rat hepatocyte before keratin reorganization (Fig. 5) (Baribault et al., 1989). EGF-induced K8 phosphorylation happens at Ser-23 of head domain and Ser-431 of tail domain (Ku and Omary, 1997).

Interleukin-6 (IL-6) significantly up-regulates K8 and K18 in intestinal epithelial cells such as Caco2-BBE (brush border expressing) cell line and IL-6 evoked K8 phosphorylation at serine residue (Fig. 5) (Wang et al., 2007b). IL-6 protect intestinal barrier via K8/K18 in compromised condition (Wang et al., 2007b).

K17, the myoepithelial keratin, is expressed in psoriasis but is not present in healthy skin (Komine et al., 1996). Increased production of interferon gamma (IFNγ) induces the expression of K17 by activating transcription factor STAT1 (Komine et al., 1996). However, it is not clear whether IFNγ induces phosphorylation and reorganization of K17.

#### 12-O-Tetradecanoylphorbol-13-acetate & LTB4

Exposure of the hepatocytes to 12-O-tetradecanoyl-phorbol-13-acetate (TPA) (150 nM), a typical activator of protein kinase C, leads to phosphorylation of K8 but not K18 (Cadrin et al., 1992). Recently, we found that transglutaminase-2 plays important role in TPA-induced K8 phosphorylation and reorganization (Fig. 6) (Lee et al., 2014). Our data show that LTB4 is an inducer of K8 phosphorylation and that ERK is involved in LTB4-induced phosphorylation and reorganization of K8 in pancreatic cancer cells. LTB4 receptor 2 (BLT2) receptor mediates effects of LTB4 via PP2A down-regulation (Fig. 5) (Park et al., 2012).

### Chemical compounds & others including physical stresses

Treatment of several human breast cancer cells including MCF7, T47D, SKBR3 with vitamin K3 (50-100 μM) leads to K8 phosphorylation at Ser-73 via MEK (MAPK/ERK kinase) 1/2 signaling (Fig. 6) (Scott et al., 2005).

Acrolein is a primary mediator of pulmonary edema and induces phosphorylation of K8 at Ser-73 in bronchiolar lung epithelia (Fig. 6) (Burcham et al., 2014).

Griseofulvin induces Mallory-Denk bodies in hepatocytes of mice (Fortier et al., 2010a). In this mice model, griseofulvin induces phosphorylation of K8 (Ser-79, Ser-436) and K18 (Ser-33) (Fig. 6).

Pervanadate, tyrosine phosphatase inhibitor, induces phosphorylation of tyrosine residue in K8, and K19, but not K18 via p38 MAP kinase (Feng et al., 1999). This process appears independent of ERK kinase pathway.

Withaferin A (WFA) binds to the vimentin and modifies perinuclear aggregates of intermediate filaments including keratin (Grin et al., 2012).

Compressive loads induce K8 phosphorylation in human disc generation by activating protein kinase C (Sun et al., 2013). Shear stress also evokes reorganization of the keratin network via the phosphorylation of K8 by PKC (Sivaramakrishnan et al., 2009). Heat stress or rotavirus infection induced phosphorylation of K8 in human colonic cell line HT29 (Liao et al., 1995).

### PHOSPHORYLATION AND REORGANIZATION OF KERATINS IN CANCER

Several reports support an active role of keratins as versatile regulators in carcinogenesis (Karantza, 2011). However, roles of phosphorylation of keratin in carcinogenesis and metastasis are controversial. For example, loss of K8 Ser-73 and Ser-431 phosphorylation is also observed in human oral squa-
Phosphorylation and Reorganization of Keratins

Epithelial-mesenchymal transition (EMT) is an important event that permit a polarized epithelial cell, to experience numerous biochemical conversions to deduce a mesenchymal phenotype of cell including increased migration, invasiveness, and significantly elevated resistance to apoptosis (Kalluri and Neilson, 2003).

Loss of keratin by phosphorylation is one of hallmarks in EMT (Kalluri and Weinberg, 2009). Therefore it is plausible that players implicated in perinuclear reorganization of keratin by phosphorylation are also involved in EMT. Accordingly, Tgase-2 involved in SPC or TPA-induced K8 phosphorylation and reorganization, is also implicated in TGF-β1-induced EMT (Park et al., 2013). ERK1/2, JNK and p38 are involved in phosphorylation of keratins and also TGF-β1-induced EMT (Park et al., 2013; Zhao et al., 2015). RKS2 involved in keratin phosphorylation, are involved in macrophage-stimulating protein-induced EMT (Ma et al., 2011).

Several phosphatases involved in dephosphorylation of keratins are also implicated in process of EMT. PRL-3 or PT-1B involved in keratin dephosphorylation also induced EMT (Liu et al., 2003). In contrast, PP2A, DEDD, and AMPK reverses EMT (Lv et al., 2012; Bhardwaj et al., 2014; Chou et al., 2014; Kim et al., 2015). Therefore, several players in keratin phosphorylation seems have an important role in EMT and new target identification in keratin phosphorylation and reorganization might be new targets for controlling EMT and metastasis.

New opportunity of compounds regulating the phosphorylation and reorganization of keratins

Modulation of keratin phosphorylation and reorganization is potential new way for controlling EMT and metastasis of cancer (Beil et al., 2003). Apparently, several kinase inhibitors including MAP kinase, might be used as agents for reducing phosphorylation and subsequent reorganization of keratins leading to EMT suppression. We attempted to identify compounds affecting the keratin phosphorylation and reorganization using SPC as inducer. We found that ethacrynic acid, a well-known diuretic, inhibits SPC-induced K8 phosphorylation, reorganization, and migration via Tgase-2 inhibition (Byun et al., 2013). We reported that BLT2 participates in the LTB4-induced K8 phosphorylation, reorganization and migration and LY255283 suppressed LTB4-induced phosphorylation and reorganization of keratins (Park et al., 2012). Therefore, BLT2 antagonists and Tgase-2 inhibitors might be new tools for controlling EMT and metastasis.

We also developed SPC blocker based on structure of SPC. Several compounds derived from SPC, suppressed SPC-induced K8 phosphorylation, reorganization and migration (Lee et al., 2014). We also screened microbial extracts and found that some microbial extracts suppress SPC-induced migration using SPC-induced migration of PANC-1 cells (Kang et al., 2011).

However, additional inducers released from tumor microenvironment that affect keratin phosphorylation and reorganization have not been identified. If several factors are released from tumor microenvironment and induced keratin phosphorylation and reorganization, blocking the common pathway would be an optimal strategy. Hence PP2A activator or inducers also might be good candidate for controlling keratin reorganization by dephosphorylating the phosphoserine residue of keratins or phosphorylated kinases (active forms) involved in phosphorylation of keratins.

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REFERENCES

Akiya, Y., Kawasaki, H., Imajoh-Ohmi, S., Fukuda, H., Ohno, S., Hirano, H., Ono, Y. and Yonekawa, H. (2007) Protein kinase C epsilon phosphatase 84K phosphorylates keratin 8 at Ser8 and Ser23 in GH4C1 cells stimulated by thyrotropin-releasing hormone. FEBS J. 274, 3270-3285.

Alam, H., Gangadaran, P., Bhate, A. V., Chaukar, D. A., Sawant, S. S., Tiwari, R., Bobade, J., Kannan, S., D'Cruze A. K., Kane, S. and Vaidya, M. M. (2011) Loss of keratin 8 phosphorylation leads to increased tumor progression and correlates with clinico-pathological parameters of OSCC patients. PLoS One 6, e27767.

Ando, S., Tokui, T., Yano, T. and Inagaki, M. (1996) Keratin 8 phosphorylation in vitro by cAMP-dependent protein kinase occurs within the amino- and carboxyl-terminal end domains. Biochem. Biophys. Res. Commun. 221, 67-71.

Barbault, H., Blouin, R., Bourgon, L. and Marceau, N. (1989) Epidermal growth factor-induced selective phosphorylation of cultured rat hepatocyte 55-kD cytokeratin before filament reorganization and DNA synthesis. J. Cell Biol. 109, 1665-1676.

Beil, M., Micoulet, A., von Wichert, G., Paschke, S., Walther, P., Om-
Gerlitz, G. and Bustin, M. (2011) The role of chromatin structure in cell migration. *Trends Cell Biol.* 21, 6-11.

Getiosis, S., Huen, A. C. and Green, K. J. (2004) Working out the strength and flexibility of desmosomes. *Nat. Rev. Mol. Cell Biol.* 5, 271-281.

Goebeler, M., Roth, J., van den Bos, C., Ader, G. and Sorg, C. (1995) Increase of calcium levels in epithelial cells induces translocation of calcium-binding proteins migration inhibitory factor-related protein 8 (MRP8) and MRP14 to keratin intermediate filaments. *Biochem. J.* 309, 419-424.

Govaere, O., Komuta, M., Berkers, J., Spee, B., Janssen, C., de Luca, F., Katozianazedeh, A., Wouters, J., van Kempen, L. C., Durnez, A., Verslype, C., De Kock, J., Rogiers, V., van Grunsven, L. A., Topal, B., Pirene, J., Vankelecom, H., Nevens, F., van den Oord, J., Pinzani, M. and Roskams, T. (2014) Keratin 19: a key role player in the invasion of human hepatocellular carcinomas. *Gut* 63, 674-685.

Green, K. J. and Simpson, C. L. (2007) Desmosomes: new perspectives on a classic. *J. Invest. Dermatol.* 127, 2499-2515.

Grin, B., Mahammad, S., Wedig, T., Cleland, M. M., Tsai, L., Herrmann, H. and Goldman, R. D. (2012) Withaferin a alters intermediate filament organization, cell shape and behavior. *PLoS One* 7, e39065.

Guo, L., Degenstein, L., Dowling, J., Yu, Q. C., Wollmann, R., Perman, B. and Fuchs, E. (1995) Gene targeting of BPG1: abnormalities in mechanical strength and cell migration in stratified epithelia and neurologic degeneration. *Cell* 81, 233-243.

Haines, R. L. and Lane, E. B. (2012) Keratins and disease at a glance. *Nat. Cell Biol.* 14, 295-302.

Hanahan, D. and Weinberg, R. A. (2011) Hallmarks of cancer: the next generation. *Cell* 144, 646-674.

Hatselli, S. and Cowin, P. (2001) Deconstructing desmoplakin. *Nat. Cell Biol.* 3, E270-272.

Hatzfeld, M. and Weber, K. (1990) The coil-coil of in vitro assembled keratin intermediate filaments is a heterodimer of type I and II keratins: use of site-specific mutagenesis and recombinant protein expression. *J. Cell Biol.* 110, 1199-1210.

He, T., Stepulak, A., Holmstrom, T. H., Omary, M. B. and Eriksson, J. E. (2002) The intermediate filament protein keratin 8 is a novel cytoplasmic substrate for c-Jun N-terminal kinase. *J. Cell Sci.* 115, 2081-2090.

Hu, X., Wu, X., Xu, J., Zhou, J., Han, X. and Guo, J. (2009) Src kinase up-regulates the ERK cascade through inactivation of protein phosphatase 2A following cerebral ischemia. *BMJ Neurosci.* 10, 74.

Kakehashi, A., Inoue, M., Wei, M., Fukushima, S. and Wmbuchi, H. (2009) Cytokeratin 8/18 overexpression and complex formation as an indicator of GST-P positive foci transformation into hepatocellular carcinomas. *Toxicol. Appl. Pharmacol.* 238, 71-79.

Kalluri, R. and Neilson, E. G. (2003) Epithelial-mesenchymal transition and its implications for fibrosis. *J. Clin. Invest.* 112, 1776-1784.

Kalluri, R. and Weinberg, R. A. (2009) The basics of epithelial-mesenchymal transition. *J. Clin. Invest.* 119, 1420-1428.

Kang, J. H., Park, M. K., Kim, H. J., Kim, Y. W., Jung, K. C., Kim, S. Y. and Lee, T. (2013) Ethacrynic acid inhibits sphingosylphosphorylcholine-mercerion and its implications for fibrosis. *Nat. Rev. Mol. Cell Biol.* 14, 233-243.

Karantza, V. (2011) Keratins in health and cancer: more than mere epithelial cell markers. *Oncoogene* 30, 127-138.

Kayser, J., Hasibekk, M., Dempfle, L., Krause, M., Grashoff, C., Buchner, J., Herrmann, H. and Bausch, A. R. (2013) The small heat shock protein Hsp27 affects assembly dynamics and structure of keratin intermediate filament networks. *Biophys. J.* 105, 1775-1785.

Khapere, N., Kundu, S. T., Sehgal, L. S., Sawant, M., Priya, R., Gosavi, P., Gupta, N., Alam, H., Karkhanis, M., Naik, N., Vaidya, M. M. and Dalal, S. N. (2012) PI3Kphospholipase C loss leads to an increase in PRL3.
levels promoting K8 dephosphorylation, which is required for transformation and metastasis. *PloS One* **7**, e38561.

Kim, E. J., Kim, H. J., Park, M. K., Kang, G. J., Byun, H. J., Lee, H. and Lee, C. H. (2015) Cardamonin suppresses TGF-beta1-induced epithelial-mesenchymal transition via restoring protein phosphatase 2A expression. *Biomed. Ther.* **23**, 141-148.

Knippel, U., Weidner, S., Zanin, N., Huber, N., Kohler, J. and Stoter, M. (2005) The casein kinase 1 family: participation in multiple cellular processes in eukaryotes. *Cell. Signal.* **17**, 675-689.

Kolsch, A., Windoffer, R. and Leube, R. E. (2009) Actin-dependent dynamics of keratin filament precursors. *Cell Motil. Cytoskel.** **66**, 976-985.

Komin, M., Freedberg, I. M. and Blumenberg, M. (1996) Regulation of epidermal expression of keratin K17 in inflammatory skin diseases. *J. Invest. Dermatol.* **107**, 569-579.

Koster, J., Geerts, D., Favre, B., Borradori, L. and Sonnenberg, A. (2003) Analysis of the interactions between BP180, BP230, plectin and the integrin alpha6beta4 important for hemidesmosome assembly. *J. Cell. Sci.* **116**, 387-399.

Kouklis, P. D., Hutton, E. and Fuchs, E. (1994) Making a connection: direct binding between keratin intermediate filaments and desmosomal proteins. *J. Cell Biol.* **127**, 1049-1060.

Koyanagi, N., Imai, T., Arii, J., Kato, A. and Kawaguchi, Y. (2014) Role of herpes simplex virus 1 Us3 in viral neuroinvasiveness. *Microbiol. Immunol.* **58**, 31-37.

Ku, N. O., Azhar, S. and Omary, M. B. (2002a) Keratin 8 phosphorylation by p38 kinase regulates cellular keratin filament reorganization: modulation by a keratin 1-like disease causing mutation. *J. Biol. Chem.* **277**, 10775-10782.

Ku, N. O., Liao, J. and Omary, M. B. (1998) Phosphorylation of human keratin 18 serine 33 regulates binding to 14-3-3 proteins. *EMBO J.* **17**, 1892-1900.

Ku, N. O., Michie, S., Resurreccion, E. Z., Broome, R. L. and Omary, M. B. (2002b) Keratin binding to 14-3-3 proteins modulates keratin filaments and hepatocyte mitotic progression. *Proc. Natl. Acad. Sci. U.S.A.* **99**, 4373-4378.

Ku, N. O. and Omary, M. B. (1997) Phosphorylation of human keratin 8 in vivo at conserved head domain serine 23 and at epidermal growth-factor-stimulated tail domain serine 451. *J. Biol. Chem.* **272**, 7556-7564.

Ku, N. O., Toivola, D. M., Strnad, P. and Omary, M. B. (2010) Cytoskeletal keratin glycosylation protects epithelial tissue from injury. *Nat Cell. Biol.* **12**, 876-885.

Ku, N. O., Zhou, X., Toivola, D. M. and Omary, M. B. (1999) The cytoskeleton of digestive epithelia in health and disease. *Am. J. Physiol. Cell Physiol.* **277**, G1108-1137.

Kuga, T., Kume, H., Kawasaki, N., Sato, M., Adachi, J., Shimizu, T., Hoshino, I., Nishimori, H., Matsubara, H. and Tomonaga, T. (2013) A novel mechanism of keratin cytoskeleton organization through casein kinase I alpha and FAM83H in colorectal cancer. *Cell. Signal.* **25**, 122-128.

Lee, C. H. (2015) Cardamonin suppresses TGF-beta1-induced epithelial-mesenchymal transition via activating selective macrophage autophagy. *Autoimmunity* **48**, 675-679.

Ma, Q., Guin, S., Padhye, S. S., Zhou, Y. Q., Zhang, R. W. and Wang, M. H. (2011) Ribosomal protein S6 kinase (RSK)-2 as a central effector molecule in RON receptor tyrosine kinase mediated epithelial to mesenchymal transition induced by macrophage-stimulating protein. *Mol. Cancer* **10**, 66.

Mellad, J. A., Warren, D. T. and Shanahan, C. M. (2011) Nesprins LINC the nucleus and cytoskeleton. *Curr. Opin. Cell Biol.* **23**, 47-54.

Menon, M. B., Schwermann, J., Singh, A. K., Franz-Wachtel, M., Pabst, O., Seidler, U., Omary, M. B., Kotlyarov, A. and Gaestel, M. (2010) p38 MAP kinase and MAPKAP kinases MK2/3 cooperatively phosphorylate epithelial keratins. *J. Biol. Chem.* **285**, 33242-33251.

Mizuzuchi, E., Samba, S., Kodama, Y. and Yokozaki, H. (2009) Down-modulation of keratin 8 phosphorylation levels by PRL-3 contributes to colorectal carcinoma progression. *Int. J. Cancer* **124**, 1802-1810.

Moll, R., Franke, W. W., Schiller, D. L., Geiger, B. and Krepler, R. (1982) The catalog of human cytokeratins: patterns of expression in normal epithelia, tumors and cultured cells. *Cell* **31**, 11-24.

Murata, T., Goshima, F., Nishizawa, Y., Daikoku, T., Takakawa, H., Ohtsuka, K., Yoshikawa, T. and Nishiyama, Y. (2002) Phosphorylation of cytokeratin 17 by herpes simplex virus type 2 US3 protein kinase. *Microbiol. Immunol.* **46**, 707-719.

Neumann, S. and Noegel, A. A. (2014) Nesprins in cell stability and migration. *Adv. Exp. Med. Biol.* **773**, 491-504.

Omary, M. B., Ku, N. O., Liao, J. and Price, D. (1998) Keratin modifications and solubility properties in epithelial cells and in vitro. *Subcell. Biochem.* **31**, 105-140.

Omary, M. B., Ku, N. O., Strnad, P. and Hanada, S. (2009) Toward unraveling the complexity of simple epithelial keratins in human disease. *J. Clin. Invest.* **119**, 1794-1805.

Omary, M. B., Ku, N. O., Tao, G. Z., Toivola, D. M. and Liao, J. (2006) "Heads and tails" of intermediate filament phosphorylation: multiple sites and functional insights. *Trends Biochem. Sci.* **31**, 383-394.

Omary, M. B., Ku, N. O. and Toivola, D. M. (2002) Keratins: guardians of the liver. *Hepatology* **35**, 251-257.

Oshima, R. G. (1982) Developmental expression of murine extraembryonic endodermal cytoskeletal proteins. *J. Biol. Chem.* **257**, 3414-3421.

Padmakumar, V. C., Libotte, T., Lu, W., Zaim, H., Abraham, S., Noegel, A. A., Gottmann, J., Foisner, R. and Karakessisoglou, I. (2005) The inner nuclear membrane protein Sun1 mediates the anchorage of Nesprin-2 to the nuclear envelope. *J. Cell Sci.* **118**, 3419-3430.

Pan, X., Hobb, R. P. and Coulombe, P. A. (2013) The expanding significance of keratin intermediate filaments in normal and diseased epithelia. *Curr. Opin. Cell Biol.* **25**, 47-56.

Pan, X., Kane, L. A., Van Eyk, J. E. and Coulombe, P. A. (2011) Type I keratin 17 protein is phosphorylated on serine 44 by p90 ribosomal protein S6 kinase 1 (RSK1) in a growth- and stress-dependent fashion. *J. Biol. Chem.* **286**, 42403-42413.

Paramio, J. M., Segrelles, C., Ruiz, S. and Jorcano, J. L. (2001) Inhibition of protein kinase B (PKB) and PKCzeta mediates keratin K10-induced cell cycle arrest. *Mol. Cell. Biol.* **21**, 7449-7459.

Park, M. K., Lee, H. J., Shin, J., Noh, M., Kim, S. Y. and Lee, C. H. (2011) Novel participation of transglutaminase-2 through c-Jun N-terminal kinase activation in spicingophosphorylcholine-induced keratin reorganization of PAN-C1 cells. *Biochim. Biophys. Acta* **1811**, 1021-1029.

Park, M. K., Park, Y., Shim, J., Lee, H. J., Kim, S. and Lee, C. H. (2012) Novel involvement of leukotriene B(4) receptor 2 through ERK activation by PP2A down-regulation in leukotriene B(4)-induced keratin phosphorylation and suppression of pancreatic cancer cells. *Biochem. Biophys. Acta* **1823**, 2120-2129.

Park, M. K., You, H. J., Lee, H. J., Kang, J. H., Oh, S. H., Kim, S. Y. and Lee, C. H. (2013) Transglutaminase-2 induces N-cadherin expression in TGF-beta1-induced epithelial mesenchymal transition via c-Jun-N-terminal kinase activation by protein phosphatase 2A down-regulation. *Eur. J. Cancer* **49**, 1692-1705.
Pavlaki, M. and Zucker, S. (2003) Matrix metalloproteinase inhibitors (MMPIs): the beginning of phase I or the termination of phase III clinical trials. *Cancer Metastasis Rev.* **22**, 177-203.

Perrig, M. D., Cairns, L., van den, I. P., Prescott, A., Hutcheson, A. M. and Quinlan, R. A. (1999) Intermediate filament interactions can be altered by HSP27 and alphab-crystallin. *J. Cell Sci.* **112** (Pt 13), 2099-2112.

Rajgor, D., Mellad, J. A., Soong, D., Rattner, J. B., Fritzler, M. J. and Scott, G. K., Atsriku, C., Kaminker, P., Held, J., Gibson, B., Baldwin, M. and Shen, J. (2014) Biofilm on keratin mediated disassembly of keratin intermediate filaments in alveolar epithelial cells. *J. Biol. Chem.* **289**, 30400-30405.

Roth, J. D. and Crompton, P. A. (2012) A wound-induced keratin inhibits Src activity during keratinocyte migration and tissue repair. *J. Cell Biol.* **197**, 381-389.

Schmidt, A. and Jager, S. (2005) Plakophilins--hard work in the desmosome. *Eur. J. Cell Biol.* **84**, 189-204.

Scott, G. K., Atsriku, C., Kaminker, P., Held, J., Gibson, B., Baldwin, M. A. and Beno, C. C. (2005) Vitamin K3 (menadione)-induced oncrosis associated with keratin 8 phosphorylation and histone H3 acetylation. *Mol. Pharmacol.* **68**, 606-615.

Selitmann, K., Roth, W., Kroger, C., Loschke, F., Lederer, M., Huttelmaier, S. and Magin, T. M. (2013) Keratins mediate localization of hemidesmosomes and repress cell motility. *J. Invest. Dermatol.* **133**, 181-190.

Sivaramakrishnan, S., Schneider, J. L., Stitikov, A., Goldman, R. D. and Ridge, K. M. (2009) Shear stress induced reorganization of the keratin intermediate filament network requires phosphorylation by protein kinase C zeta. *Mol. Cell. Biol.* **29**, 2757-2765.

Snedmer, T. N. and Omary, M. B. (2014) Post-translational modifications of intermediate filament proteins: mechanisms and functions. *Nat. Rev. Mol. Cell Biol.* **15**, 163-177.

Snider, N. T., Park, H. and Omary, M. B. (2013) A conserved rod domain phosphotyrosine that is targeted by the phosphatase PTP1B promotes keratin 8 protein insolubility and filament organization. *J. Biol. Chem.* **288**, 31329-31337.

Snider, N. T., Weerasinghe, S. V., Iniguez-Lluhi, J. A., Herrmann, H. and Omary, M. B. (2011) Keratin hypersumoylation alters filament dynamics and is a marker for human liver disease and keratin mutation. *J. Biol. Chem.* **286**, 2279-2284.

Sonnenberg, A. and Liem, R. K. (2007) Plakins in development and disease. *Exp. Cell Res.* **313**, 2189-2203.

Sripuranth, B., Vaidya, M. M. and Kalraiya, R. D. (2010) O-GlcNAcylation determines the solubility, filament organization, and stability of keratins 8 and 18. *J. Biol. Chem.* **285**, 34062-34071.

Steinert, P. M. (1988) The dynamic phosphorylation of the human intermediate filament keratin 1 chain. *J. Biol. Chem.* **263**, 13333-13339.

Stroka, K. M. and Konstantopoulos, K. (2014) Physical biology in cancer. 4. Physical cues guide tumor cell adhesion and migration. *Am. J. Physiol. Cell Physiol.* **306**, C98-C109.

Sugimoto, M., Inoko, A., Shiromizu, T., Nakayama, M., Zou, P., Yone-mura, S., Hayashi, Y., Izawa, I., Sasoh, M., Uji, Y., Kaibuchi, K., Kiyono, T. and Inagaki, M. (2008) The keratin-binding protein AIB1 regulates polarization of epithelial cells. *J. Cell Biol.* **183**, 19-28.

Sun, Z., Guo, Y. S., Yan, S. J., Wan, Z. Y., Gao, B., Wang, L., Liu, Z. H., Gao, Y., Samartzis, D., Lan, L. F., Wang, H. Q. and Luo, Z. J. (2013) CK8 phosphorylation induced by compressive loads underlies the downregulation of CK8 in human disc degeneration by activating protein kinase C. *Lab. Invest.* **93**, 1323-1330.

Suozzi, K. C., Wu, X. and Fuchs, E. (2012) Spectraplakin: master orchestrators of cytoskeletal dynamics. *Cell Biol. Chem.* **197**, 465-475.

Suresh, S. (2007) Biomechanics and biophysics of cancer cells. *Acta Biomech.* **3**, 413-438.

Tao, G. Z., Toivola, D. M., Zhou, Q., Stmad, P., Xu, B., Michie, S. A. and Omary, M. B. (2006) Protein phosphatase-2A associates with and dephosphorylates keratin 8 after hypoxsmotic stress in a site- and cell-specific manner. *Cell Biol. Sci.* **119**, 1425-1432.

Toivola, D. M., Boor, P., Alam, C. and Stmad, P. (2013) Keratins in health and disease. *Curr. Opin. Cell Biol.* **25**, 91-98.

Toivola, D. M., Goldman, R. D., Garrod, D. R. and Eriksson, J. E. (1997) Protein phosphatases maintain the organization and structural interactions of hepatic keratin intermediate filaments. *J. Cell Sci.* **110**, 23-33.

Toivola, D. M., Zhou, Q., Engish, L. S. and Omary, M. B. (2002) Type II keratins are phosphorylated on unique motif during stress and mitosis in tissues and cultured cells. *Mol. Biol. Cell* **13**, 1857-1870.

Velasco, G., Gomez del Pulgar, T., Carling, D. and Guzman, M. (1998) Evidence that the AMP-activated protein kinase stimulates rat liver carnitine palmitoyltransferase I by phosphorylating cytoskeletal components. FEBS Lett. **439**, 317-320.

Wang, H., Quah, S. Y., Dong, J. M., Mander, E., Tang, J. P. and Zeng, Q. (2007) PRL-3 down-regulates PTEN expression and signals through PI3K to promote epithelial-mesenchymal transition. *Cancer Res.* **67**, 2922-2926.

Wang, L., Sinivasan, S., Theiss, A. L., Merlin, D. and Sitarman, S. V. (2007b) Intereukin-6 induces keratin expression in intestinal epithelial cells: potential role of keratin-8 in interleukin-6-induced barrier function alterations. *J. Biol. Chem.* **282**, 8219-8227.

Wang, Q., Griffin, H., Southern, S., Jackson, D., Martin, A., McIntosh, P., Davy, C., Masterson, P. J., Walker, P. A., Laskey, P., Omary, M. B. and Doorbar, J. (2004) Functional analysis of the human papillomavirus type 16 E1-E4 protein provides a mechanism for in vivo and in vitro keratin filament reorganization. *J. Virol.* **78**, 821-833.

Windoffer, R., Beil, M., Magin, T. M. and Leube, R. E. (2011) Cytoskeleton in motion: the dynamics of keratin intermediate filaments in epithelia. *J. Cell Biol.* **194**, 669-678.

Wolf, S., Windoffer, R. and Leube, R. E. (2005) Dissection of keratin dynamics: different contributions of the actin and microtubule systems. *Eur. J. Cell Biol.* **84**, 311-328.

Wolf, S., Windoffer, R. and Leube, R. E. (2007) p38 MAPK-dependent shaping of the keratin cytoskeleton in cultured cells. *J. Cell Biol.* **177**, 795-807.

Yano, T., Tokui, T., Nishi, Y., Nishizawa, K., Shibata, M., Kikuchi, K., Tsuki, S., Yamauchi, T. and Inagaki, M. (1991) Phosphorylation of keratin intermediate filaments by protein kinase C, by calmodulin-dependent protein kinase and by cAMP-dependent protein kinase. *Eur. J. Biochem.* **197**, 281-290.

Zhao, L., Geng, H., Liang, Z. F., Zhang, Z. Q., Zhang, T., Yu, X. and Zhong, C. Y. (2015) Benzidine induces epithelial-mesenchymal transition in human uroepithelial cells through ERK1/2 pathway. *Biochim. Biophys. Res. Commun.* **459**, 643-649.

Zhao, Q., Cadrin, M., Herrmann, H., Chen, C. H., Chalkley, R. J., Burlingame, A. L. and Omary, M. B. (2006) Keratin 20 serine 13 phosphorylation is a stress and intestinal goblet cell marker. *J. Biol. Chem.* **281**, 16453-16461.

Zhou, Q., Snider, N. T., Liao, J., Li, D. H., Hong, A., Ku, N. O., Cartwright, C. A. and Omary, M. B. (2010) Characterization of in vivo keratin 19 phosphorylation on tyrosine-391. *PLoS One* **5**, e13538.

Zhou, X., Liao, J., Hu, L., Feng, L. and Omary, M. B. (1999) Characterization of the major physiologic phosphorylation site of human keratin 19 and its role in filament organization. *J. Biol. Chem.* **274**, 12861-12866.

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