Phosphorylation and Reorganization of Keratin Networks: Implications for Carcinogenesis and Epithelial Mesenchymal Transition

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Abstract
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

INTRODUCTION
Metastasis is critical hallmark of cancer and contributes to the 90% of cancer death (Hanahan and Weinberg, 2011). Diverse approaches have been attempted to combat the metastasis of cancer. The spot light has been on matrix metalloproteinase inhibitors but the clinical outcome of matrix metalloproteinase inhibitors in most cancer metastasis is poor (Coussens et al., 2002; Pavlaki and Zucker, 2003).

Recently, several researchers investigated physical properties of cancer cells and found that metastatic cancer cells are significantly softer than other benign or normal cells (Cross et al., 2007). This softness of metastatic cancer cells might be useful as diagnostic marker. Measures of physical properties might also be useful as assay methods for new compounds modulating the physical properties of cancer cells using novel devices such as optical stretcher, optical tweezer, and atomic force microscopy (Suresh, 2007).

Because the physical properties and mechanotransduction of cancer cells are crucial in various steps of the metastatic process, control of physical properties of cancer cell may be an effective therapeutic approach for patients suffering cancer (Stroka and Konstantopoulos, 2014).

However, measuring changes of physical properties of cancer cells is not easy to most researchers in pharmacology fields. We are interested in the biological phenomena reflecting the changes of physical properties such as keratin reorganization via phosphorylation, which is changed by sphingosylphosphorylcholine (SPC) and related to viscoelasticity of metastatic cancer cells (Beil et al., 2003). We have studied the underlying molecular mechanisms in keratin 8 (K8) phosphorylation and perinuclear reorganizations of cancer cells for several years. We have reviewed the results of these studies together with the relevant literature.

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**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).

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 anti-parallel 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 the 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-

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**Table 1. Expression of keratin proteins in epithelial tissues**

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

*Modified from Haines and Lanes, and Loschke (Haines and Lane, 2012; Loschke et al., 2015).
nuclear keratin network and are linked to desmosome and hemidesmosome (Fig. 3) (Windoffer et al., 2011).

KERATIN IN THE EPITHELIAL CELLS

In the epithelium 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 isofom; 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 Lane (Fuchs and Raghavan, 2002; Gerlitz and Bustin, 2011; Haines and Lane, 2012).

**Linking to desmosome 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 (Gestis 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 nucleat envelope**

Lamins underlie the inner face of nuclear membrane and also make stable structures within the nucleus interior which contains emerin, lamin B-receptor, and SUN (Sad1 and UNC84 domain containing) 1/2 (Friedl et al., 2011). Nesprins belong to a family of proteins that are mainly known for their position along the nuclear envelope (Mellad et al., 2011).

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, Syne 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
B4 (LTB4)-evoked K8 phosphorylation and reorganization and increased solubility of keratin in the cytoplasm (Omary due of keratin leads to disintegration of the stable structure (Snider and Omary, 2014). Phosphorylation of serine residue by phosphorylation is a special event for metastatic cancer cells. However, it is not yet clear that perinuclear reorganization of keratin proteins induced by shear stress (Sivaramakrishnan et al., 1996). Phosphorylation of 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 K8 leading to reorganization of keratin proteins induced by shear stress (Sivaramakrishnan et al., 2009). Phosphorylation of Ser-73 of K20 is increased after PKC activation but it is not clear whether PKC phosphorylates Ser-13 of K20 (Menon et al., 2010). MK2 also phosphorylates Ser-13 of K20 (Menon et al., 2010).

**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., 2002a; 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 Ca2+-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 thyrotropin-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 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.

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

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

*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 Thr308 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 Iα
Casein kinase Iα (CK-Iα) plays an essential role in the phosphorylation and degradation of β-catenin (Knippschild et al., 2005). Casein kinase Iα (CK-Iα) mediates FAM83H (family with sequence similarity 83 member H)-dependent reorganization of keratin filaments (Kuga et al., 2013). Inhibition of CK-Iα 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-Iα. Ser-73 and Ser-431 of K8 and Ser-33 and Ser-52 of K18 are not candidates of substrates of CK-Iα (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 K6α and K6β (Rotty and Coulombe, 2012).

Miscellaneous kinases
The AMP-activated protein kinase (AMPK) is important in the biological response induced by metabolic changes and is involved in SPC-induced K8 phosphorylation (Fig. 5) (Pan et al., 2011). 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 phosphorylated phospho ERK and phospho JNK (Fig. 5) (He et al., 2002; Hu et al., 2009). PP2A directly dephosphorylates K8 during hyposmotic 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 (DEDD), 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) (Goebeler 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) (Perrg 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-
mos cell carcinoma (OSCC) tissues evaluated by immuno-
histochemistry, in which dephosphorylation greatly associated
with size, and progression of the tumor (Alam et al., 2011).
Moreover, overexpression of K8 and K18 is related to up-regu-
lation of histone type 2 H2aα3 and keratin reorganization may
accelerate cancerous transformation of glutathione S-trans-
ferase P-form positive foci in the course of rat hepatocarcino-
genesis (Kakehashi et al., 2009). Similarly, K19 expression in
human hepatocellular carcinoma is correlated with increased
invasiveness and metastasis (Govaere et al., 2014).

On the other hand, loss of K8 and K18 leads to increased
collective emigration and invasiveness of breast cancer cells
(Fortier et al., 2010a). Similarly, SPC evokes a perinuclear re-
organization of keratin proteins via phosphorylation of Ser-431
of K8, and increased migration of human pancreatic cancer
cells (Beil et al., 2003). JNK and ERK phosphorylates K8 at
Ser-431, and stimulate the perinuclear reorganization of ker-
atin resulting enhanced migration (Busch et al., 2012; Park et
al., 2011).

The probable differences in results might be by use of dif-
ferent kinds of cells and methods (Windoffer et al., 2011).

Epithelial-mesenchymal transition (EMT) is an important
event that permit a polarized epithelial cell, to experience nu-
merous 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 ker-
atin by phosphorylation are also involved in EMT. Accordingly,
Tgase-2 involved in SPC or TPA-induced K8 phosphoryla-
tion and reorganization, is also implicated in TGF-β-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 ker-
inin 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-
P1B involved in keratin dephosphorylation also induced EMT
(Wang et al., 2007a; Hiraga et al., 2013). 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 com-
ounds affecting the keratin phosphorylation and reorganiza-
tion using SPC as inducer. We found that ethacrynic acid, a
well-known diuretic, inhibits SPC-induced K8 phosphoryla-
tion, 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-in-
duced 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 microen-
vironment that affect keratin phosphorylation and reorganiza-
tion have not been identified. If several factors are released
from tumor microenvironment and induced keratin phosphor-
ylation and reorganization, blocking the common pathway
would be an optimal strategy. Hence PP2A activator or induc-
ers also might be good candidate for controlling keratin reor-
ganization by dephosphorylating the phosphoserine residue
of keratins or phosphorylated kinases (active forms) involved
in phosphorylation of keratins.

CONCLUSION

Metastatic cancer cell is much softer than non-metastatic
cancer cells (Cross et al., 2007). Viscoelasticity of cancer cells
is related to keratin architecture. So elucidating new players
to regulate the keratin phosphorylation and reorganization might
provide new targets for suppressing the metastasis. Further-
more, novel compounds modulating the phosphorylation and
reorganization of keratin might be a new hope for fighting
against metastasis of cancer.

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