



Review article

Keratin intermediate filaments in the colon: guardians of epithelial homeostasis

Lauri Polari^a, Catharina M. Alam^a, Joel H. Nyström^a, Taina Heikkilä^a, Mina Tayyab^a, Sarah Baghestani^a, Diana M. Toivola^{a,b,*}

^a Cell Biology, Biosciences, Faculty of Science and Engineering, Åbo Akademi University, Turku, Finland

^b Turku Center for Disease Modeling, University of Turku, Finland



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ABSTRACT

Keratin intermediate filament proteins are major cytoskeletal components of the mammalian simple layered columnar epithelium in the gastrointestinal tract. Human colon crypt epithelial cells express keratins 18, 19 and 20 as the major type I keratins, and keratin 8 as the type II keratin. Keratin expression patterns vary between species, and mouse colonocytes express keratin 7 as a second type II keratin. Colonic keratin patterns change during cell differentiation, such that K20 increases in the more differentiated crypt cells closer to the central lumen. Keratins provide a structural and mechanical scaffold to support cellular stability, integrity and stress protection in this rapidly regenerating tissue. They participate in central colonocyte processes including barrier function, ion transport, differentiation, proliferation and inflammatory signaling. The cell-specific keratin compositions in different epithelial tissues has allowed for the utilization of keratin-based diagnostic methods. Since the keratin expression pattern in tumors often resembles that in the primary tissue, it can be used to recognize metastases of colonic origin. This review focuses on recent findings on the biological functions of mammalian colon epithelial keratins obtained from pivotal *in vivo* models. We also discuss the diagnostic value of keratins in chronic colonic disease and known keratin alterations in colon pathologies. This review describes the biochemical properties of keratins and their molecular actions in colonic epithelial cells and highlights diagnostic data in colorectal cancer and inflammatory bowel disease patients, which may facilitate the recognition of disease subtypes and the establishment of personal therapies in the future.

1. Introduction

Keratins belong to the intermediate filament (IF) protein family and are major components of the cellular cytoskeleton, which supports cell structure and tissue homeostasis. The understanding and classification of keratin proteins have evolved over time, as in the 19th and early 20th century keratins were simply referred to as “insoluble filamentous proteins” (Rouse and Van Dyke, 2010). It was already at that time suggested that keratins are present in skin and appendages, such as hair, and in tissue epithelia (Bailey, 1921; Barritt et al., 1930). Later, keratins were split into hard and soft keratins, followed by classification according to chemical properties and, finally, by amino acid sequence (Moll et al., 1982a; Majumdar et al., 2012). The current and most recently updated nomenclature of keratin genes (*KRT*) and proteins was published in 2006 (Schweizer et al., 2006).

The keratin content is highest in appendages and skin, as suggested by the name of epidermal cells, keratinocytes. Nevertheless, the keratin concentration is also relatively high in simple epithelia, making up 0.2 %, 0.3 % and 0.5 % of the total tissue protein in mouse liver, pancreas and small intestine, respectively (Zhong et al., 2004). The scientific interest in epithelial keratins grew as it was discovered that keratins and their circulating fragments can be used as markers of the origin and growth of various epithelial cancers and metastasis due to tissue-specific keratin expression patterns (Moll et al., 1982b).

In this review, we discuss the current knowledge of intestinal epithelial keratins, with focus on mammalian colonic keratins and their function in colon health, as well as the utilization of keratins as colonic disease markers in clinical diagnostics. Several detailed and excellent reviews have been published on related topics, including intestinal intermediate filament proteins in non-mammalian organisms, in recent

* Corresponding authors at: Cell Biology/Biosciences, Faculty of Science and Engineering, Åbo Akademi University, BioCity, Tykistökatu 6A, FIN-20520, Turku, Finland.

E-mail addresses: lauri.polari@abo.fi (L. Polari), diana.toivola@abo.fi (D.M. Toivola).

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years (Majumdar et al., 2012; Coch and Leube, 2016; Omary, 2017; Salas et al., 2016; Strnad et al., 2016).

2. Keratin intermediate filaments and intestinal epithelial cells

Keratins are encoded by 54 epithelial and hair-related genes in humans and belong to a conserved protein family of over 70 IF proteins, which are divided into six different IF types. In addition to keratins the IF family includes vimentin, desmin, glial fibrillary acidic protein, neurofilaments, nestin, synemin, lamins, phakinin and filensin (Kim and Coulombe, 2007; Eriksson et al., 2009). IF and keratin protein expression depends on the cell/tissue type (Fig. 1A) and the differentiation level (Kim and Coulombe, 2007; Strnad et al., 2008). These proteins are the building blocks of mechanically strong structural filaments, which also possess many other dynamic functions (Magin et al., 2007; Snider and Omary, 2014). Owing to the variety and distribution of IFs and known IF mutations in different tissues, over 110 human diseases have been associated with IFs (www.interfil.org).

2.1. The structure and regulation of keratins

Keratins (K) comprise the type I acidic (K9–K28 and K31–K40) and II neutral/basic (K1–K8, K71–K80 and K81–K86) IF proteins (Schweizer et al., 2006; Godsel et al., 2008), which form obligate heteropolymers consisting of at least one protein of each type (Ku and Omary, 2000; Omary et al., 2004). Keratin filaments are non-polar and, as the other IFs, assemble through the intercoiling of protein subunits that are made up of a central coiled-coil α -helical rod domain accompanied by flanking non- α -helical head and tail domains (Herrmann et al., 2009). The keratin head (N-terminal) and tail (C-terminal) domains differ in length (Fig. 1B) and contain the motifs which confer most of the molecular diversity observed in this protein family (Coulombe and Omary, 2002; Omary et al., 2009). Type I and II keratin heterodimers then form hetero-tetramers via anti-parallel hydrophobic binding between rod domains, leading to formation of unit-length keratin filaments (ULF). As the precursors of short filaments, ULFs are then elongated and finally form the long keratin filaments (Köster et al., 2015; Herrmann and Aebi, 2016; Eldirany et al., 2019). The spontaneous self-assembly of keratin filaments is independent of protein synthesis and instead driven by a continuous release of non-filamentous subunits, which subsequently reattach to filaments Kölsch et al., 2010. Interestingly, two common keratin polymers composed of K5-K14 and K8-K18 dimers share surprisingly similar mechanical and chemical properties. However, when these pairs were mismatched to K5-K18 and K8-K14 polymers, their biophysical properties were different to both each other and also to the naturally occurring pairs (Yamada et al., 2002). This emphasizes that different keratins are not identical in their functionality, while their roles as structural proteins may be more universal.

2.2. Simple epithelial keratins

The expression patterns of type I and II keratins also vary in a cell type-specific manner (Strnad et al., 2008). For example, stratified epithelia in the skin express K5/K14 and/or K1/K10 pairs, depending on the differentiation state of the cell (Moll et al., 1982a; Coulombe and Omary, 2002). Simple epithelia in organs such as the liver, lung, intestine and pancreas express mainly the type II simple epithelial keratins (SEK) K8 and K7, paired with type I K18, K19 and K20 (Fig. 1A) (Coulombe and Omary, 2002; Moll et al., 2008; Omary et al., 2009). As constituents of the cytoskeleton, keratin IFs are involved in supporting cellular mechanical integrity and tissue stability. In epithelial cells, the filament spatial organization is characterized by filament bundles connecting to neighboring cells via desmosomes and to the extracellular matrix via hemidesmosomes (Waschke, 2008). Accordingly, it has been shown that keratin mutations are associated with tissue fragility in liver and skin-blistering disorders, collectively known as keratinopathies.

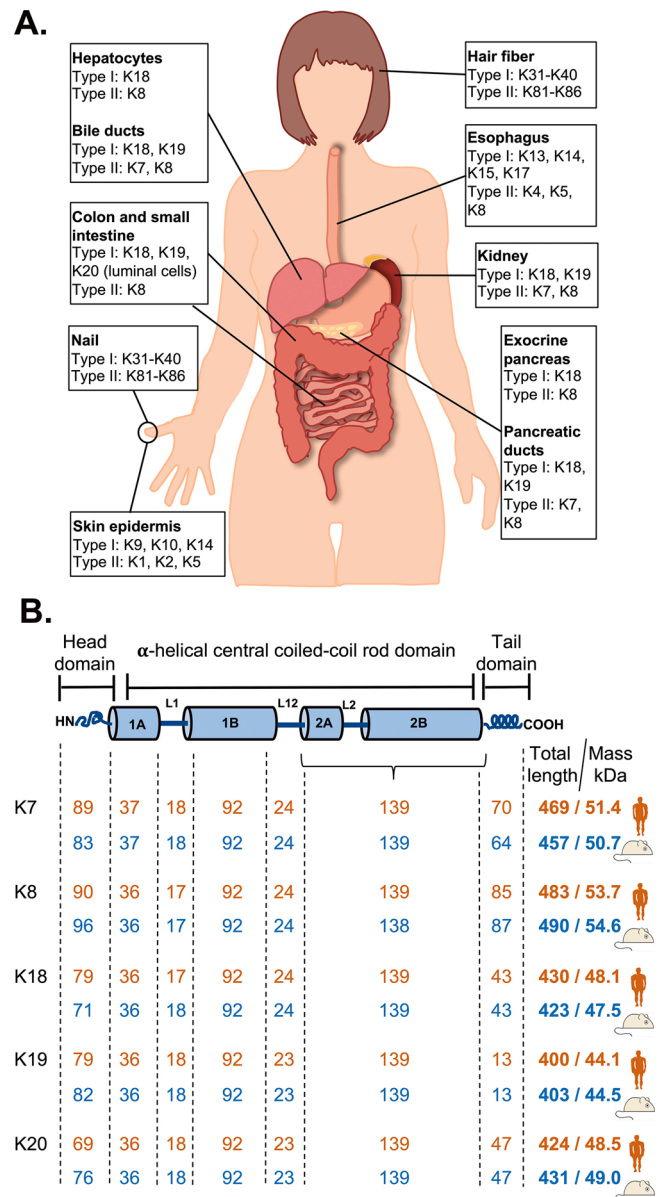


Fig. 1. Major keratin family member expression patterns in selected human epithelial tissues under basal conditions and the keratin protein structural domain organization and molecular weights in human and mouse. (A) Keratin expression patterns are diverse and varied between tissues and epithelial cell types. A schematic illustration of the major Type I and Type II keratins expressed in human intestine, esophagus, liver, pancreas, kidney, skin, hair and nail are shown. Note that keratins expressed at minor levels or those in other organisms are not shown (adapted from Moll et al., 2008; Omary et al., 2009; Salas et al., 2016; and Singh et al., 2009; Toivola et al., 2010; Bouameur and Magin, 2017).

(B) The illustration shows a generalized representation of the keratin protein structure with structural domain organization (adapted from Herrmann et al., 2009). 1A, 1B, 2A and 2B represent the four sub-helices present in the coiled-coil domain. L1, L2 and L12 are linker segments between the coils. Below the keratin structure, the length of the different domains is indicated as the number of amino acids in both man (brown) and mouse (blue) for K7, K8, K18, K19, and K20 (head domains also include the initiator methionine as the first amino acid). The head and tail domains exhibit most of the variation between keratin proteins as well as between species, while the coiled-coil and linker parts are more conserved. Sequence data from www.uniprot.org.

Human keratinopathies are in many cases phenocopied in keratin-deficient animal models (Loranger et al., 1997; Omary et al., 2009; Loschke et al., 2015).

Several keratin functions are effectuated through keratin-associated proteins (Green et al., 2005; Kim and Coulombe, 2007), including cytolinker, anchoring, enzymatic, apoptosis-related and adaptor proteins (Strnad et al., 2008). Keratins respond to extra- and intracellular stimuli by upregulation or adjustment of the resident or de-novo keratin levels (Zhong et al., 2004; Wang et al., 2007; Toivola et al., 2010). Keratins also undergo rapid posttranslational modifications, such as phosphorylation, glycosylation, acetylation and sumoylation (Omary et al., 2009), which has been comprehensively reviewed (Majumdar et al., 2012; Snider and Omary, 2014). These modifications and keratin interactions with keratin-binding proteins regulate filament assembly/disassembly, and consequently often affect filament solubility and dynamics (Omary et al., 2006; Kölsch et al., 2010; Snider and Omary, 2014).

2.3. Keratin expression in the colon epithelium

The innermost layer of the mammalian intestinal tissue consists of a single layer of columnar epithelial cells, which make up the folds of the colonic crypts and the finger-like villi and crypts in the small intestine. These rapidly regenerating structures consist of differentiated cells including enterocytes, goblet cells, Paneth cells (in the small intestine) and enteroendocrine cells, which are all derived from intestinal stem cells located in the bottom of the crypts (Umar, 2010). While the detailed keratin expression pattern in all differentiated cell types has not been carefully scrutinized with cell-type specific markers, most colonic and small intestinal epithelial cells share a similar keratin expression pattern throughout the intestinal epithelium comprising of the major keratins K8 and K19, and to a lesser extent K18 (Fig. 2A, B) (Chu and Weiss, 2002). However, in the mouse small intestine, K18 is expressed most strongly in the lower part of the crypts and in scattered goblet cells in the upper part of the villus (Zhou et al., 2003). K20 is expressed at very low levels in the bottom of the colon crypt (Fig. 2), but increases significantly in the differentiated luminal cells (Moll et al., 1992; Calnek and Quaroni, 1993; Yun et al., 2000; Zhou et al., 2003). In contrast, K7, which is basally expressed in the mouse intestine, localizes mostly to the lower and middle parts of the crypt (Zhou et al., 2003; Sandilands et al., 2013; Asghar et al., 2015). In human colon, K7 is below detection limits, but upregulated in some colon tumors (see Chapter 3.1). Finally, a few

studies report that type I keratins K23 (Rogers et al., 2004; Birkenkamp-Demtroder et al., 2007), K24 (Sprecher et al., 2002) as well as type II K80 (Langbein et al., 2010; Li et al., 2018) are weakly expressed in the colon. Interestingly, a recent study suggests that the type I keratin K15, found in progenitor cells of complex epithelia such as hair follicles, is also expressed in mouse intestinal stem cells (Giroux et al., 2018) (Fig. 2). SEK are also expressed in colon mesothelium together with vimentin (LaRocca and Rheinwald, 1984).

2.4. Keratins and colon cell differentiation

Terminal differentiation affects the keratin expression pattern in enterocytes, similar to epidermal cells. It is hence possible that keratins are modulators of the differentiation process. The cell fate of colonic epithelial cells is regulated by various signaling pathways, such as Wnt, BMP and Notch, which are central cascades in stem cell proliferation and differentiation (Umar, 2010; Koch, 2017). Especially Notch-1 has recently been associated with keratin-mediated changes in epithelial cell differentiation (Lähdeniemi et al., 2017; Saha et al., 2019). Notch-1 expression is dependent on keratin expression, as colonic K8 deficiency leads to decreased expression of both the full-length Notch-1 receptor and the Notch-1 intracellular domain, as well as downstream target genes. K8 knockout-induced Notch-1 deactivation moreover correlates with a cell fate shift from enterocytes toward goblet and enteroendocrine cells (Lähdeniemi et al., 2017), which is accompanied by hyperproliferation of the colon epithelium (Toivola et al., 2004). Similarly, K19 silencing downregulates Notch-associated genes and reduces the expression of stemness markers in colon cancer cells *in vitro* (Saha et al., 2019), implicating that K19 promotes cell differentiation. Moreover, both the loss of K15 in intestinal stem cells (Giroux et al., 2018) and reduced K8 expression in K8^{+/-} mice (Liu et al., 2017; Asghar et al., 2015) impair epithelial regeneration. Taken together, these findings suggest that keratins may be required at multiple stages for maintaining the balance between differentiation and proliferation processes, especially in the colonic epithelium.

3. Functions of keratins in colon epithelial cells – lessons from transgenic mice and *in vitro* studies

Several transgenic animal models with SEK anomalies develop liver disorders, such as hepatitis and diet/drug-induced liver injury, which phenocopy corresponding human diseases (Bouameur and Magin, 2017;

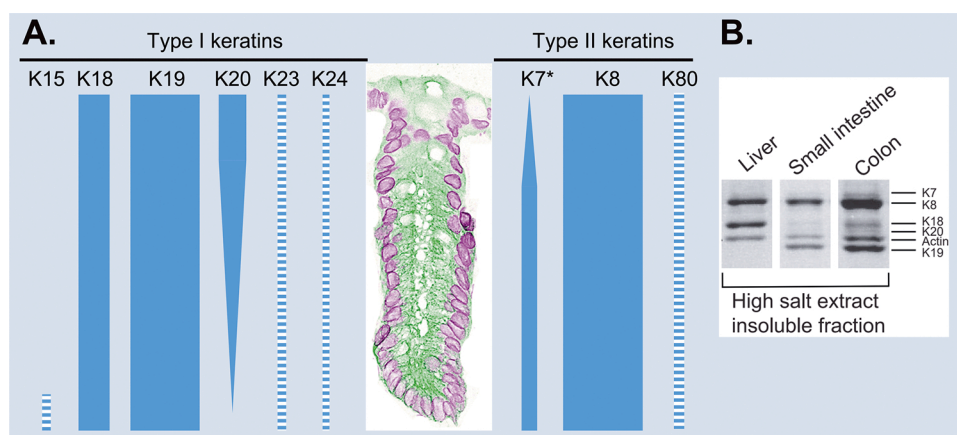


Fig. 2. Relative expression of keratins in colonic crypts. (A) The columns represent type I and type II keratins expressed in human and mouse colonic crypts. The columns represent keratin distribution on the crypt bottom to crypt top axis, and the thickness of each column represents the relative amount of each keratin in the crypt. Striped columns indicate less studied keratins, for which the actual expression pattern in crypts still needs to be confirmed. The crypt image is from a healthy mouse colon, immunostained for K8 (green) and nuclear lamin A (magenta). K7* is expressed in mouse, but not human colon. (B) Keratin expression patterns and molecular weight differences in epithelial tissues can be visualized by high salt extraction of insoluble keratins (Ku et al., 2004; Strnad et al., 2016), followed by separation with SDS-PAGE and staining with Coomassie brilliant blue. Keratin expression in mouse liver (only K8/K18 in hepatocytes), small intestine and colon (K8/K19 as major keratins, and K7/K18/K20 as minor) including actin, can be seen as indicated.

Yi et al., 2018). Similarly, keratin-deficient mouse models (Fig. 3) have provided a better understanding of the functions of epithelial keratins and their role in the pathology of intestinal diseases. K8 was the first IF protein knocked out in the mouse ($K8^{-/-}$) (Baribault et al., 1993), and it has been followed by additional transgenic SEK knockout mice (Fig. 3) as well as mice expressing human disease-related keratin mutations (Yi et al., 2018). However, thus far, mice with other keratin deficiencies than K8 or humanized keratin mutations have less drastic or non-existent colon phenotypes compared to $K8^{-/-}$ mice (Fig. 3), highlighting the importance of K8 as the major type II keratin in colonic epithelia.

3.1. K8 maintains colonic homeostasis by protecting the colon from inflammation and colorectal cancer

One of the first evidence for a physiological disease-associated role of keratins in the colon came from the K8-null mice, which displayed a colitis-like phenotype with damaged colonic epithelium, hyperplastic lesions and rectal prolapse (Baribault et al., 1994). The colonic inflammation in K8-null mice is associated with an increase of Th2-type cytokines, infiltration of CD4+ T cells and expression of major histocompatibility complex antigens (Habtezion et al., 2005). The heterozygous K8-null mice suffer a 50 % loss of K8, however, the remaining keratins protect them from spontaneous inflammation (Asghar et al., 2015), although these mice are more susceptible to dextran sodium sulphate (DSS)-induced experimental colitis than wild-type mice. Furthermore, they have significantly taller crypts, which are still shorter than crypts in $K8^{-/-}$ mice (Asghar et al., 2015; Liu et al., 2017). This suggests that the proliferative phenotype is dependent on keratin levels rather than inflammation. A genome-wide microarray analysis of isolated colonic epithelial cells showed that $K8^{-/-}$ colonocytes are resistant to apoptosis in a gutmicrobe-dependent manner, as evidenced by

upregulation of survivin and β 4-integrin-mediated pFAK activation (Habtezion et al., 2011). Both K8-null and heterozygous mice are prone to lipopolysaccharide (LPS)-induced nuclear factor kappa B (NF- κ B) activation. Furthermore, tumor necrosis factor-associated factor 6 (TRAF6), which acts as a mediator of Toll-like receptor (TLR)-based signaling, was found to be highly ubiquitinated and hence activated in $K8^{-/-}$ mouse colon tissue (Dong et al., 2016). K8-null colonocytes showed a 2.5-fold increase of TLR9, which could contribute to the colitis-induced colorectal cancer (CRC) and NF- κ B activation seen in these mice (Habtezion et al., 2011; Misiorek et al., 2016; Liu et al., 2017; Luo et al., 2020).

The protective role of keratins in the colon together with the observation of several hallmarks of cancer in $K8^{-/-}$ mice suggests that K8 might participate in tumor suppression. Indeed, even though $K8^{-/-}$ mice do not develop CRC spontaneously, they developed significantly more tumors in the distal colon compared to controls in two experimental models (treatment with the colon carcinogen azoxymethane (AOM) or crossing with tumor-susceptible $Apc^{Min/+}$ mice) (Misiorek et al., 2016). Similarly, heterozygous K8-null mice challenged with DSS/AOM developed significantly more tumors throughout the colon compared to controls (Liu et al., 2017). Colon tumorigenesis is linked to the activation of the interleukin-22 (IL-22) pathway via downregulation of the inhibitory IL-22 binding protein (IL-22BP) (Huber et al., 2012; Mizoguchi et al., 2018). Intriguingly, in K8-null mice the IL-22 pathway is highly activated by a complete loss of IL-22BP, leading to increased JAK/STAT signaling (Misiorek et al., 2016) which stimulates colonocyte cell proliferation, tissue repair, and tumorigenesis (Moniruzzaman et al., 2019). Keratins may provide protection from inflammation via their putative interaction with the inflammasome, and this may limit its activity (Misiorek et al., 2016).

In contrast to K8-null mice, K7- (Sandilands et al., 2013), K18- (Magin et al., 1998) and K19-null (Tamai et al., 2000) mice have no obvious intestinal phenotypes (Fig. 3). Additionally, transgenic mice expressing human K18 or K20 filament-disrupting point mutations (K18 R90C or K20 R80 H) similarly had no obvious intestinal phenotype, while the intestine tolerates the overexpression of wild-type human K8, K18 and K20 well (Ku et al., 1995; Zhou et al., 2003). Mice with knockout of both K18 and K19 are embryo-lethal, possibly due to mechanical fragility (Hesse et al., 2000; Hesse et al., 2005), as are mice lacking the entire keratin multiprotein family (Vijayaraj et al., 2009). Together, these results promote the idea that type I keratins K18 and K19 can replace each other, while the presence of one of them is necessary for filament stability and function. To this end, only a single K18 allele fully rescued the K18 and K19 double-negative embryo lethal phenotype (Hesse et al., 2007). Furthermore, $K15^{-/-}$ mice showed poor intestinal recovery after radiation injury (Giroux et al., 2018), and a similar phenomenon was seen in the esophagi of $K15^{-/-}$ mice (Giroux et al., 2017). This suggests that K15 may preserve the regenerative capacity of the progenitor cells, as it is not found in differentiated epithelial cells.

3.2. Keratins associate with membrane junctions and maintain the intestinal barrier

The intestinal epithelium is composed of a tightly regulated barrier, which is maintained through specialized cellular junctions, and the maintenance of these are vital for epithelial health in the intestine (Brooke et al., 2012). Dysregulation of cell junctions in the gut leads to compromised gut barrier properties, which is thought to be a major factor in inflammatory and autoimmune intestinal diseases, and even in some food allergies (Groschwitz and Hogan, 2009). Although the keratin filament networks extend throughout the cytoplasm of the cell, intestinal keratins are found highly concentrated in the apical region under the actin-rich microvillae and on the lateral sides of the cells (Salas et al., 2016), as seen in K8-YFP knock-in mice (Schwarz et al., 2015). An association between keratins and the lateral desmosomes was

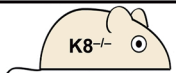



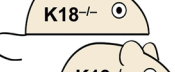
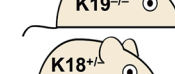
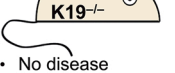
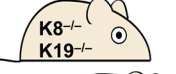
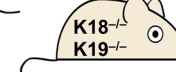
 <ul style="list-style-type: none"> • 50-90 % embryo lethality • Hyperproliferation/ increased crypt length/prolapse • Ion transport defects and diarrhea • Protein mistargeting • Resistant to apoptosis • Increased permeability • Chronic inflammation • Susceptible to experimental CRC 	 <ul style="list-style-type: none"> • Hyperproliferation/ increased crypt length • Ion transport defects • Susceptible to experimental colitis • Susceptible to colitis-induced CRC 	
<p>Baribault et al., 1993, 1994; Toivola et al., 2004; Habtezion et al., 2005, 2011; Asghar et al., 2015, 2016; Misiorek et al., 2016</p>	<p>Asghar et al., 2015; Lähdeniemi et al., 2017; Liu et al., 2017</p>	
 <ul style="list-style-type: none"> • Impaired crypt regeneration 	    <ul style="list-style-type: none"> • No disease phenotype in colon 	  <ul style="list-style-type: none"> • 100 % embryo lethality • Overall fragility
<p>Giroux et al., 2017, 2018</p>	<p>Magin et al., 1998; Harada et al., 1999; Hesse et al., 2007; Sandilands et al., 2013</p>	<p>Hesse et al., 2000; Tamai et al., 2000</p>

Fig. 3. Transgenic SEK knockout mouse models and their main colon disease phenotypes. Susceptibility indicates increased susceptibility compared to respective wild-type keratin expressing mice. CRC = colorectal cancer.

demonstrated in the early 90's by the binding of keratins to desmoplakin in skin cells (Kouklis et al., 1994). Since then, a number of studies have shown that epidermal and simple epithelial keratins are linked to desmosomal cadherins (Kouklis et al., 1994; Moch et al., 2020) through several other desmosome-associated proteins, in particular plakins, plakophilin and plakoglobin (Coch and Leube, 2016). Keratins also connect to the extracellular matrix on the basal side of colonocytes by binding to the linker protein plectin at hemidesmosomes and $\alpha6\beta4$ integrins. Mutation of integrin $\alpha6$ leads to a nearly identical colonic disease phenotype as in K8-null mice, suggesting that both proteins are important at the basal side of the colonocyte (De Arcangelis et al., 2017). In the small intestine, the keratin-binding protein trichoplein and K8/K18 co-localize at the apical junctional domain (Nishizawa et al., 2005). Trichoplein also co-localizes with desmoplakin and may thus be a component of the desmosome in the intestine (Nishizawa et al., 2005). The desmoplakin-keratin interaction has proven important for desmosomal localization and mechanical support in keratinocytes, however in the intestine, desmoplakin does not appear to be essential for cell adhesion per se, nor for keratin localization at the apical region of the epithelial cell membrane (Sumigray and Lechler, 2012). This apical intestinal keratin network is likely functioning in close collaboration with the actin network, possibly via the actin-binding protein plastin and K19 (Grimm-Gunter et al., 2009). Furthermore, F-actin together with other membrane markers exhibit a patchy distribution in K8-deficient surface colonocytes (Toivola et al., 2004).

K8 knockout leads to mildly impaired intestinal barrier properties *in vivo* (Misiorek et al., 2016), and downregulation of K8 (in K8 heterozygote animals) exacerbates the increase in colon permeability in response to experimental DSS-induced colitis (Liu et al., 2017). An *in vitro* study using monolayers of a colon cancer cell line expressing human K8/K18 disease mutations (K8 G62C, K8 K464N and K18 S230T) showed a 30 % increase in paracellular permeability and altered distribution of claudin-4 and ZO-1 tight junction proteins compared with colonocytes expressing wild-type K8/K18 (Zupancic et al., 2014). This would suggest that K8/K18 play a role in maintaining colon barrier properties through interaction with tight junction proteins. However, despite the aforementioned evidence, the colonic diarrhea phenotype in K8 knockout mice may ultimately not depend directly on disrupted tight junctions, but on interfered electrolyte transport. While tight junction permeability and paracellular transport was normal in K8 knockout distal colon epithelium mounted *ex vivo* on Ussing chambers, significant deficiencies in electrolyte transport caused by mistargeting of ion channel proteins were observed in the absence of K8 (Toivola et al., 2004).

3.3. Keratins help target apical ion transport and membrane proteins

The intestine efficiently absorbs and secretes electrolytes and water mainly through chloride and sodium transporters and osmosis (Field, 2003). Epithelial cell polarity and microfilament- and microtubule-mediated maintenance of such polarity are important for vectorial ion transport (Höfer et al., 1998). K8^{-/-} mouse colonic epithelium analyzed *ex vivo* displayed decreased short circuit current with decreased net Na and Cl ion absorption, which increases the water content in stool and causes mild diarrhea. This imbalanced ion transport in the colon begins at an early age in K8^{-/-} mice, and the mistargeting of the ion transporter AE1/2 (Toivola et al., 2004; Habtezion et al., 2005) was normalized in mice treated with broad-spectrum antibiotics after weaning, suggesting the involvement of the colonic microbiota in this phenotype (Habtezion et al., 2011).

Studies of ulcerative colitis (UC) patients show that the expression of chloride transporter downregulated by adenoma (DRA) expression is decreased during inflammation (Yang et al., 1998). Similar to K8^{-/-} mice, DRA^{-/-} mice develop diarrhea, which is linked to decreased chloride exchange with a compensatory increase in potassium- and/or sodium-absorbing transporters, leading to excess chloride in stool

(Schweinfest et al., 2006). The diarrheic phenotype in K8^{-/-} mice was further supported by the loss of DRA expression in K8^{-/-} distal colon and in Caco-2 cells lacking K8. Since K8^{+/-} mice showed a partial decrease in sodium and chloride ion transport (Toivola et al., 2004) and in DRA levels (Asghar et al., 2016), it would suggest a more direct role of keratins in these processes.

K8 deletion causes mistargeting of apical membrane proteins in the small intestine, as evidenced by the mistargeting of the Cl⁻ channel cystic fibrosis transmembrane conductance regulator (CFTR) in small intestinal villi cells (Ameen et al., 2001), while a normal apical localization of CFTR was observed in crypt cells likely due to the expression of K7 in these cells. CFTR has been shown to bind K18, and this binding increased the apical recycling rate of CFTR, leading to increased cell surface expression of CFTR (Duan et al., 2012). Correspondingly, CFTR cell surface expression was decreased in the duodenal epithelium of K18^{-/-} mice (Duan et al., 2012). In humans, CFTR mutations may induce cystic fibrosis (Guilbault et al., 2007), which is associated with a higher risk of developing gastrointestinal cancer and obstruction of the intestine (Strubberg et al., 2018). Interestingly, K8 deletion leads to an intestinal phenotype similar to CFTR deficiency, which includes diarrhea and colitis (Guilbault et al., 2007; Toivola et al., 2004; Baribault et al., 1994). Whether the targeting of ion transporters and other membrane proteins (Toivola et al., 2004; Helenius et al., 2015; Salas et al., 2016) is directly dependent on keratins or other cytoskeletal components remains to be investigated.

3.4. Keratins and the colonic microbiota regulate colonocyte energy metabolism

Colon homeostasis is maintained through complex interplay between the epithelial cells and the microbiota, immune system and stroma. In addition to the previously described perturbations in barrier function and ion transport, the lack of keratins in colonic epithelial cells have been linked to alterations in the microbiota and in epithelial cell metabolism. This concept was first supported by evidence from a genome-wide analysis of colonocytes from wild-type and K8-null mice, in which the most differentially upregulated genes in K8-null mice were normalized after the mice were treated with broad-spectrum antibiotics (Habtezion et al., 2011). The microbiota contributes to colonic homeostasis through the generation of bacterial metabolites, such as short chain fatty acids. Of these, butyrate constitutes the primary energy source for colonic epithelial cells (Roediger, 1980). Butyrate is suggested to possess anti-inflammatory, anti-carcinogenic and barrier-strengthening properties (Hamer et al., 2008; Venegas et al., 2019).

The K8^{-/-} intestinal microbiota is composed of fewer microorganisms than K8^{+/+} and K8^{+/-} microbiotas (Liu et al., 2017; Habtezion et al., 2011). It could be hypothesized that the microbiota is partly flushed out due to the diarrhea and/or eliminated by the increased immunological activity observed in K8^{-/-} colon (Asghar et al., 2016; Misiorek et al., 2016). Conversely, the microbiota may participate in keratin regulation, for example as seen by increased K8 expression in human colon adenocarcinoma HT-29 cells co-cultured with *Bifidobacterium breve* (Sánchez et al., 2015), or increased keratin expression in porcine ileum mucosa infected with *Salmonella* Typhimurium (Collado-Romero et al., 2012). In contrast, prolonged *Salmonella* infection in pigs elicited keratin downregulation (Arce et al., 2014). Intriguingly, keratins have been implied as a mediator of *Salmonella* invasion into eukaryotic cells (Carlson et al., 2002).

Keratins are also involved in colonic epithelial cell metabolism (Helenius et al., 2015). K8 levels are lower in colorectal tumors in patients with high fecal butyrate concentrations (Khan et al., 2011). Similarly, the loss of K8 in colon epithelium correlates with increased fecal levels of butyrate, and decreased levels of the major butyrate transporter MCT1 in colonic epithelial cells (Helenius et al., 2015; Hadjiagapiou et al., 2000). No significant changes in the ratio of the

major butyrate-producing *Firmicutes* were seen in K8^{-/-} mice (Helenius et al., 2015; Liu et al., 2017), suggesting a defect in butyrate uptake. Further down-stream, the ketogenic response is blunted, and the rate of ketogenesis is diminished, as seen by downregulation of the rate-limiting enzyme of mitochondrial ketogenesis, HMGCS2 (Hegardt, 1999), and its transcriptional regulator PPAR α . Aging male K8^{-/-} mice also produce anti-mitochondrial autoantibodies against HMGCS2 (Toivola et al., 2015). Lastly, K8^{-/-} colonic epithelial cell mitochondria have fewer cristae than their wild-type counterparts (Helenius et al., 2015). The mitochondrial energy metabolism phenotype in K8^{-/-} is intriguing, as keratins and other IFs, including vimentin and desmin, have been associated with the regulation of mitochondrial morphology, function and energy metabolism in various cell types, including β -cells, skin keratinocytes, fibroblasts and muscle cells (Silvander et al., 2017; Steen et al., 2020; Matveeva et al., 2015; Milner et al., 2000). The exact molecular mechanisms for how keratins regulate energy metabolism in the colon remain to be elucidated, however these mechanisms likely involve keratin-mediated targeting and/or stabilization of proteins and organelles (Silvander et al., 2017; Steen et al., 2020; Toivola et al., 2005; Schwarz and Leube, 2016; Lehman et al., 2020).

In addition to the observations made in K8^{-/-} mice, several studies have linked changes in SCFAs, inflammation and/or CRC to changes in keratins and energy metabolism. Intriguingly, patients with non-inflamed UC exhibit increased K19 levels, while the levels of K8 and several tricarboxylic acid cycle, oxidative phosphorylation and fatty acid synthesis proteins are decreased (Moriggi et al., 2017). Conversely, K8 and K18 levels were decreased and metabolism was shifted towards polyamine and carnitine biosynthesis in non-inflamed Crohn's disease patients (Moriggi et al., 2017). Decreased keratin levels have also been observed in colonic adenomas as well as in healthy subjects with low fecal butyrate concentrations (Evans et al., 2015), while fiber intake and consequent SCFA production restored keratin levels (Evans et al., 2015). Furthermore, SCFA treatment of HCT-116 CRC cells elicited an upregulation of K18, K19 and proteins involved in glycolysis and oxidative phosphorylation (Kilner et al., 2012). As colonic inflammation, CRC and colonic metabolism (SCFA) exert changes on keratins, and keratins are involved in inflammatory, cancer and metabolic pathways, keratins are emerging as critical integrators of these complex pathways.

3.5. Stress responses modulate colonic keratins

The stress-responsive nature of keratins has been well-demonstrated in multiple organs including skin (Moll et al., 1982a, 1982b; Jin and Wang, 2014), liver (Nakamichi et al., 2005; Guldiken et al., 2015; Szabo et al., 2015; Guldiken et al., 2016), pancreas (Wögenstein et al., 2014; Zhong et al., 2004), kidney (Djudjaj et al., 2016) and lung (Sivar-amakrishnan et al., 2009). In the human colon (see Section 3.2) and murine colon, stress-inducing factors such as inflammation, aging or microbiota affected the expression of keratins as well as their post-translational modifications (Helenius et al., 2016). Induced acute colitis in mice (DSS) upregulated K7, K19 and K20 levels and increased phosphorylation on the stress and mitosis-inducible K8 phosphorylation site serine 74 (K8 pS74) (Helenius et al., 2016) and on K20 S13 (Zhou et al., 2006). Interestingly, the K20 S13 site is highly phosphorylated in goblet cells under basal conditions, and further elevated upon starvation-induced mucin secretion (Zhou et al., 2006). K7 and K20 were also upregulated in chronic DSS-induced colitis, whereas K8, K18 and K19 were unaltered (Helenius et al., 2016). Interestingly, also non-disease conditions, such as broad-spectrum antibiotics and aging, upregulated colonic keratins. Antibiotic treatment started around the time of weaning in young mice increased K8, K19 and K8 pS74. Conversely, in 14 months old mice, proximal colon K8, K18 and K20 levels were increased, while the distal colon exhibited more K7 and K19 when compared to 3 month old mice (Helenius et al., 2016). While SEK are mostly phosphorylated on serine residues, K19 tyrosine 391 phosphorylation may occur during stress in the colon (Zhou et al., 2010). In

conclusion, stress conditions induce a moderate keratin upregulation and/or hyperphosphorylation in the colon, and the different types of stress induce different sets of keratins. The stress-induced keratin upregulation occurs in many tissues at the transcriptional level and during the regenerative phase after injury, suggesting that keratins are important in tissue repair (Toivola et al., 2010). IL-6 may be a contributing factor since IL-6 induces K8/K18 expression in intestinal cells, further supporting the idea that keratins maintain barrier functions in stress situations (Wang et al., 2007)

4. Keratins as diagnostic biochemicals in human colonic diseases

Since keratin expression in the colonic epithelium varies according to cell type and extent of differentiation, it is not surprising that alterations in keratin expression have been detected in chronic colon diseases, most notably in colon cancer. The keratin expression pattern in carcinomas and their metastases (Table 1) is in general considered homologous with the tissues of origin, as expression patterns rarely possess major variability during carcinogenesis, invasion and metastasis. In 1982, Moll and coworkers suggested that keratin expression patterns can be used to track down the anatomical and cellular origin of tumors and metastases (Moll et al., 1982a; Moll et al., 1982b). Since then, several diagnostic methods based on keratin quantitation, e.g. by immunohistochemistry and real-time PCR, have been proposed and some are routinely used by pathologists to identify intestinal cancers. Proteomics studies of human colon neoplasms have shown that among several proteins, especially keratins are altered (Evans et al., 2015). Furthermore, recent findings suggest that alterations in keratin expression patterns can be associated with specific subtypes of colon disease, such as UC-induced cancer or microsatellite-stable colon tumors.

4.1. Simple epithelial keratins as markers for colon cancer

K8, K18, K19 and K20, the most abundant keratins in healthy human colonic epithelium, have been found in the majority of colon tumors and in their metastases (Moll et al., 1992; Omary et al., 2009). However, many earlier studies did not differentiate between specific keratins as they utilized pan-keratin antibodies, e.g. tissue polypeptide antigen (TPA) recognizing K8, K18 and K19. TPA was widely used as a prognostic epithelial tumor and tumor metastasis marker, that could also be used to detect circulating keratin fragments and keratin-expressing cells in bodily fluids (Rasmuson et al., 1984; Carpelan-Holmström et al., 1996; Barak et al., 2004). Keratin levels in bodily fluids are low in healthy individuals, but are known to soar in patients with epithelial cell carcinomas (Barak et al., 2004; Dandachi et al., 2005), which has been associated with poor survival of cancer patients (Rasmuson et al., 1984; Mishaeli et al., 1998). Despite the multiple single keratin-specific antibodies currently available, various pan-keratin antibodies and their mixtures, such as AE1 (type I keratins K10, K14, K15, K16, K19) and AE3 (type II keratins K1-K8), are still used as diagnostic tools. As they stain cells of epithelial origin, they help to recognize tumor structure, invasion and budding in the colon (Yamada et al., 2017; Rieger et al., 2017; Mehta et al., 2018).

4.1.1. Keratins 20 and 7

K20 expression has been linked to the highly differentiated cellular state of tumors, as its average expression in healthy colon is the most intense in the terminally differentiated cells next to the lumen (Fig. 4), while no K20 has been detected in the stem cell area in the bottom of the crypts (Moll et al., 1992; Yun et al., 2000). Significant K20 expression in metastases has been used as a clinical biomarker to determine the primary site of circulating cancer cells or metastases, as many other common adenocarcinomas, such as those of breast, prostate, lung and ovary, rarely express K20 (Moll et al., 1992; Chu et al., 2000; Kummar et al., 2002; Kende et al., 2003). Nevertheless, some colon adenocarcinomas

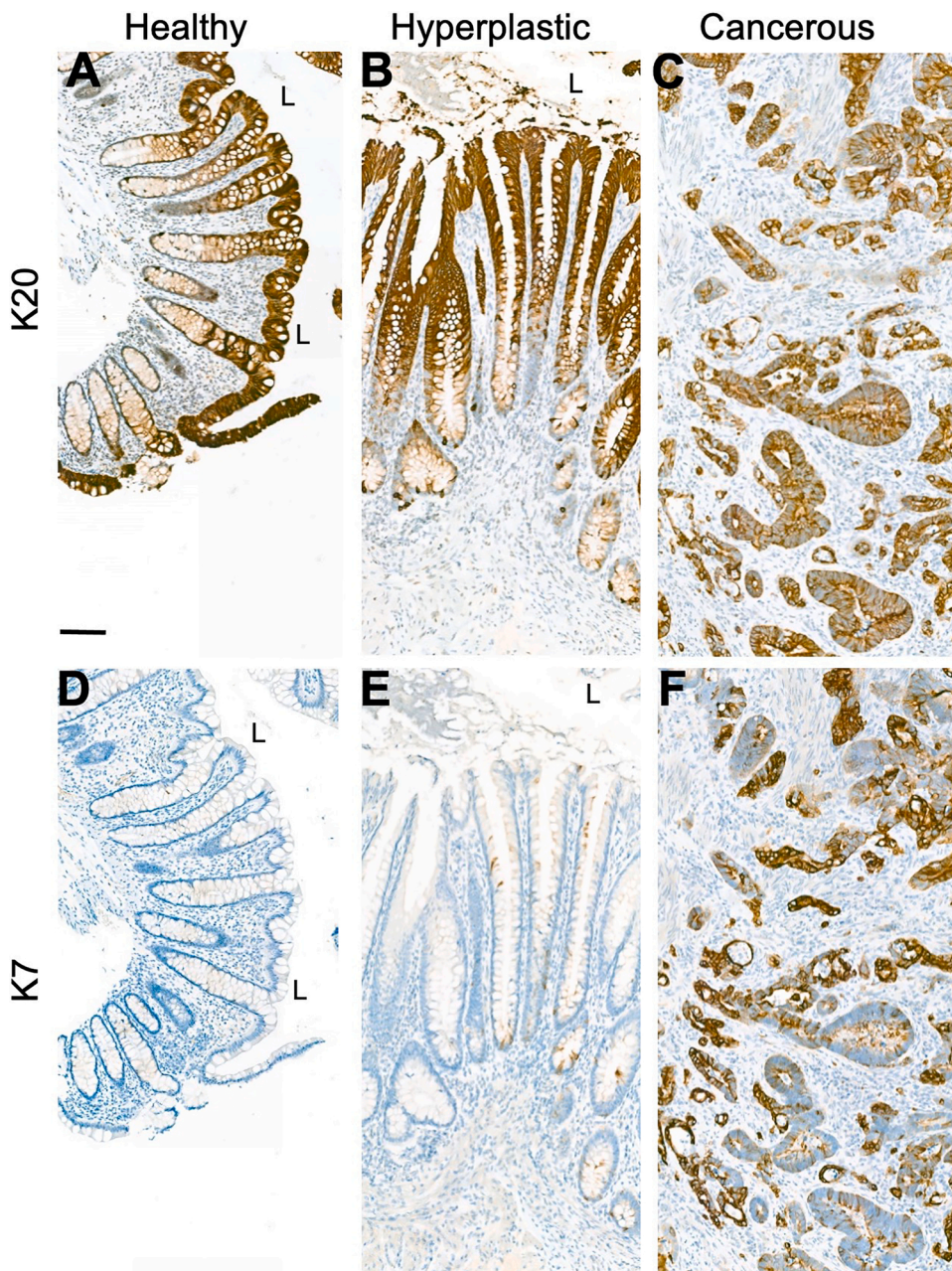


Fig. 4. K7 and K20 are expressed differently in the healthy and diseased human colon epithelium. K20 expression is shown in (A) healthy colon epithelium, (B) hyperplastic lesions and (C) malignant (cancerous) colon tissue from UC patients. K7 expression is shown in (D) healthy colon epithelium, (E) hyperplastic lesions and (F) malignant (cancerous) colon tissue from the same patients as shown for K20. Blue color is hematoxylin counter stain, L = lumen. Scale bar in A for all images =100 μ m.

do not express significant amounts of K20, especially poorly differentiated tumors, which are often K20-negative (Moll et al., 1992; Yamagishi et al., 2013; Kim et al., 2013). This phenomenon may possibly occur if these tumors originate from the K20-negative lower crypt proliferative compartment, or from cells that have dedifferentiated. Among high grade right-sided tumors, located in the proximal part of the colon (Iacopetta, 2002), K20 negativity was more common than in left-sided tumors located in distal parts of the colon (Park et al., 2002; Kende et al., 2003; Kim et al., 2013). Similarly, the K20-negative phenotype was more common in tumor cells with an increased length of nucleotide sequences (microsatellites, MSI) (McGregor et al., 2004; Lugli et al., 2008). Colon tumors with microsatellite instability generally exhibit a lower differentiation stage than microsatellite-stable (MSS) tumors, supporting the finding that the lack of K20 expression is associated with a poor differentiation stage of tumors (Moll et al., 1992). Previous findings support each other, as MSI tumors in the colon are more often right-sided (Baran et al., 2018). While significant variation between

tumors exists, approximately 73-93 % of CRC cases possess at least partial K20-positivity (Table 1). Technical issues, such as the use of different antibodies and variable cutoff values for K20-positivity, are likely reasons for the deviation between studies.

K20 expression in circulating cancer cells also occurs in CRC, but was not associated with any of the clinicopathological parameters of CRC subtypes, except advanced tumor stage (Wang et al., 2006; Shen et al., 2008). It is intriguing that surgical tumor resection may in a fact increase the number of K20-positive circulating cells a week after resection (Šamija et al., 2013). In a longer follow-up after resection, K20 expression in serum samples had an inverse correlation with the postoperative survival rate (Li et al., 2015). While circulating K20 might be an actual prognostic marker for post-surgical tumor burden, cautiousness is still needed when interpreting the results, as studies assessing the presence of K20 in peripheral blood of CRC patients show major variations in their results (Bustin et al., 1999; Schuster et al., 2004; Wang et al., 2006; Shen et al., 2008; Šamija et al., 2013)

Table 1
Percentage of colon cancers expressing specific keratins in different studies.

Cancer	K7+	K20+	K7-/K20+	Other K+	Patients	Reference
Colon adenocarcinoma	10 %	85 %			40	Wang et al., 1995
Colon adenocarcinomas *				K23 90 % £ K23 36 %££	55	Birkenkamp-Demtroder et al., 2007
Colon adenocarcinoma		97 %			93	Moll et al., 1992
Colon adenocarcinoma	5 %	100 %			20	Chu et al., 2000
Colon adenocarcinoma	16 %	97 %			77	Loy and Calaluce, 1994
Colorectal adenocarcinoma	19 %	79 %			52	Gurzu and Jung, 2012
Large intestine adenocarcinoma	17 %	93 %	79 %		29	Kende et al., 2003
Colorectal carcinoma	10 %	85 %			205	Landau et al., 2014
Colorectal carcinoma	9 %	73 %	68 %		225	Park et al., 2002
Colorectal carcinoma	9 %	93 %	83 %		1197	Lugli et al., 2008
Colorectal carcinoma	30 %	86 %	87 %		264	Yamagishi et al., 2013
Colorectal carcinoma	16 %	80 %	66 %		44	McGregor et al., 2004
Metastatic and primary colon carcinomas	34 %	88 %		K8 100 %	32	Wauters et al., 1995
Invasive adenocarcinoma	14 %	80 %	76 %		263	Hernandez et al., 2005
Primary tumors and metastasis of CRC *	7 %	79 %		K8 85 % K18 96 % K19 84 % K5 3 % K14 60 %	468	Knösel et al., 2006
Metastatic adenocarcinoma			100 % 88 % €		26	Kummar et al., 2002
Primary CRC	17 %	81 %	66 %		196	Bayrak et al., 2011
Primary CRC *	up			K8 100 % K80 60 %	40	Li et al., 2018
Primary CRC	9 %				370	Harbaum et al. 2011
Colorectal cancers ***		up		K8 up K19 down	19	Polley et al., 2006
Colorectal cancer *				K18 100 %	108	Zhang et al., 2019
Colorectal cancer *, **		74 % 77 % \$			62	Li et al., 2015
Colorectal cancer *				K23 up	17	Gao and Yang, 2020
Sporadic CRC *		up			39	Tunca et al., 2013
UC, associated dysplasia and cancer	45 %	100 %			51	Stenling et al., 2007
UC-associated and sporadic colon neoplasm	70 %# 16 % ##	88 %# 92 %##			91	Tatsumi et al., 2006
Colorectal carcinoma and LN metastasis	7 %				214	Czapiewski et al., 2016
Rectal adenocarcinoma	13 %	100 %	87 %		30	Ramalingam et al., 2001
Microsatellite unstable colorectal cancer		83 %			109	Kim et al., 2013
Poorly differentiated adenocarcinoma in colon and rectum	32 %	58 %			156	Imai et al., 2014

The percentage of colon tumors positive (+) or negative (-) for K7, K20 or other SEK are shown as indicated. Patient numbers and cancer subtype names are adapted from original research articles as indicated. The results are based on immunohistochemistry, except in cases marked with * for RT-PCR, ** for ELISA, or *** for proteomics. Numbers marked with € indicating metastasis; #UC-induced; ## sporadic; \$ mRNA; £ MSS; ££ MSI.

A majority of K20-positive intestinal carcinomas and metaplasia do not express K7, and K7-negativity can be considered as an additional marker for cancers of intestinal origin (Loy and Calaluce, 1994; Wauters et al., 1995; Wang et al., 1995; Park et al., 2002; Harbaum et al., 2011). This common phenotype has made the combination of K20 and K7 analyses a valid marker of cancers of colonic origin (Table 1), as it is rare to nonexistent in many common adenocarcinomas, such as those of lung, breast, urothelium, liver and ovary (Tot, 2002; Karantza, 2011). Of the different CRC types, well and moderately differentiated adenocarcinomas, mucinous adenocarcinoma and signet ring cell carcinoma most frequently exhibited a K7-negative and K20-positive phenotype (Bayrak et al., 2011; Yamagishi et al., 2013). The lack of K7 expression (Fig. 4) is no surprise, as K7 is not usually found in healthy intestinal epithelia and considered a marker of ductal differentiation (Ormsby et al., 1999). Nevertheless, focal K7 expression is sometimes found in normal colon mucosa, especially close to cancerous tissue (Gurzu and Jung, 2012). While the majority of colon carcinomas are K7-negative, multiple studies have found patchy or sporadic K7 expression in 7-17 % of colon and rectal tumors (Table 1) (Ramalingam et al., 2001; Park et al., 2002; Hernandez et al., 2005; Tatsumi et al., 2006; Bayrak et al., 2011; Harbaum et al., 2011; Landau et al., 2014; Czapiewski et al., 2016).

K7 is strongly expressed in anal glands (Williams et al., 1995) and overlying squamous mucosa (Ramalingam et al., 2001) and, consequently, it is a possibility that K7-positive colon tumors were actually anal gland adenocarcinomas. However, this is unlikely as anal gland

tumors are exceedingly rare and colon tumors often are not located near the anal gland area (Ramalingam et al., 2001). No association between K7-positivity and patient age or gender has been found (Park et al., 2002; Hernandez et al., 2005), however, K7-positivity was 4-6 times more frequent in *BRAF*-mutated CRC compared to *BRAF* wild-type tumors (Gurzu and Jung, 2012; Landau et al., 2014). In addition, K7 expression was more common in high-grade colon carcinomas and when the tumor was right-sided (Park et al., 2002; Harbaum et al., 2011). It was especially common in tumors induced by UC, as 45-70 % of those were K7-positive (Tatsumi et al., 2006; Stenling et al., 2007). Among particular tumor areas, K7 expression was most common in the outermost parts of invasive tumors, budding areas and lymph node metastases, indicating that it may promote invasion (Bayrak et al., 2011; Harbaum et al., 2011). The K7 status of primary tumors has no clear effect on the survival of colon adenocarcinoma patients, while K7-positivity in lymph node metastases indicates an adverse prognosis (Imai et al., 2014; Czapiewski et al., 2016).

4.1.2. Keratin 8, 18 and 19

While pan-keratin antibodies, such as TPA recognizing K8, K18 and K19, have been used to quantify circulating CRC-derived cells (Rasmussen et al., 1984; Mellerick et al., 1990), limited data is available regarding the expression of other keratins besides K7 and K20 in CRC. K18 has been implicated as an independent prognostic factor for poor survival in advanced CRC (Carpelan-Holmström et al., 1996). Likewise,

K18 upregulation in primary tumors was an unfavorable prognostic marker for survival (Zhang et al., 2019). K18 serum concentrations were generally increased in CRC patients, and there is a trend for increasing K18 concentrations from early to advanced stages of CRC (Greystoke et al., 2012; Sirmio et al., 2020). Circulating K18 levels also correlate with systemic inflammation markers, especially IL-6 and CXCL8 (Sirmio et al., 2020). It was recently hypothesized that overexpressed K18 might act as an actual oncogene in CRC by promoting cell proliferation and invasion (Zhang et al., 2019). Additionally, high post-surgical levels of circulating caspase-cleaved K18 fragments (M30) were associated with earlier cancer recurrence, possibly indicating systemic residual tumor load similar to K20 (Ausch et al., 2009).

Increased K8 expression is associated with tumor burden, as K8 expression increases in both polyp and cancer mucosa (Wauters et al., 1995; Polley et al., 2006), and K8 degradation fragments have been suggested to accumulate in CRC tissue (Nishibori et al., 1996). However, K8 downregulation in CRC, accompanied by a K20-negative phenotype, was linked to an aggressive phenotype and suggested to indicate epithelial to mesenchymal transition (Knösel et al., 2006). Loss of K8 phosphorylation was also suggested to promote tumor migration and formation of metastasis (Mizuuchi et al., 2009).

K19 is, similar to K8 and K18, expressed in most CRC types (Chu and Weiss, 2002; Knösel et al., 2006), and K19 levels increase in peripheral blood of CRC patients (Wang et al., 2006) and in fecal samples (Chang et al., 2009; Yang et al., 2010), suggesting a role for K19 as a tumor burden marker. K19 is often studied by detecting its soluble fragments in serum using an antigen-based CYFRA 21-1 assay (Pujol et al., 1993; Dohmoto et al., 2001). CYFRA 21-1 has been utilized as a prognostic marker for cancers, especially lung cancers, as multiple carcinomas besides CRC are characterized by circulating K19 subunits. CYFRA 21-1 is also elevated in CRC patients (Holdenrieder et al., 2012), and slightly increased in benign diseases, such as colon polyps and adenomas (Dressen et al., 2017; Lim et al., 2018).

4.1.3. Keratin 23 and 24

K23 and K24 are among the most recently characterized simple epithelial keratins (Zhang et al., 2001; Sprecher et al., 2002). They are normally weakly expressed in colon (Rogers et al., 2004), while K23 was found to be upregulated in intestinal adenocarcinomas, in lymph nodes and in liver metastases (Birkenkamp-Demtroder et al., 2007; Gao and Yang, 2020). K24 is suggested to be upregulated in early-onset CRC (Hong et al., 2007), while K23 expression is significantly more pronounced in microsatellite-stable tumors (Kim et al., 2004; Birkenkamp-Demtroder et al., 2007) than in instable ones, suggesting the utilization of K23 as a differentiation marker of CRC tumor subtypes. Overexpression of K23 was associated with worse survival of CRC patients (Zhang et al., 2017).

4.2. Keratins in inflammatory bowel disease

According to the experimental data from murine models discussed above, keratins may possess multiple roles in colon diseases, especially in colitis and CRC. In contrast to CRC, keratins are rarely used as biomarkers for inflammatory colon diseases, as the loss of epithelium in acute disease and the epithelial proliferation in the regenerative phase indirectly affects keratin levels and should be taken into account. However, acute inflammation downregulates the expression of colonic keratins, including K8, K18, K19, and K20 (Stenling et al., 2007), while K8, K18 and K19 were upregulated in longstanding pan-colitis (Corfe et al., 2015) (see also Section 2.4). On the contrary, K7 expression increases in actively inflamed areas and UC-associated neoplasms as shown in a few studies (Fig. 4) (Tatsumi et al., 2006; Stenling et al., 2007). K7 expression in IBD might thus be indicative of neoplastic development.

Chronic UC induces local genomic instability in the colon (Cottliar et al., 2000; Wanders et al., 2020), and it can be hypothesized that

keratins might be among the mutated genes, especially as keratin mutations have clearly been linked as susceptibility factors to liver diseases (Omary et al., 2009). A few studies have addressed keratin mutations in IBD, and while a few patients with K8 variants were found (Owens et al., 2004), no significant evidence of K8, K18 or K19 mutations associated with IBD has been reported (Owens et al., 2004; Tao et al., 2007). In addition, no association between the common K8 mutations Y54H or G62C and IBD was found (Büning et al., 2004). Therefore, there is a need for more research focusing on the expression of keratins in IBD and their role in disease etiology. Finally, in addition to IBD and CRC, there are other relatively common intestinal diseases, such as diverticulitis, appendicitis and microscopic colitis, in which potential keratin alterations remain unexplored.

5. Conclusions

The role for keratins in tumor diagnosis is useful and well-established in clinics where K7-negativity and K20-positivity can guide oncologists to the origin of metastases. Circulating keratins and their fragments are also potential markers for tumor burden. In the future, the novel molecular knowledge gained from mouse studies will be helpful to understand the effects of keratin changes in colonic diseases, to recognize dysregulated keratin-related cell signaling and to facilitate the development of personal therapies accordingly. The poorly understood role of keratins in the multifactorial inflammatory colon diseases and their disease etiology warrants a closer look also at disease sub-types.

Evidence from mouse models show that K8 is, similar to the human colon, the single most important keratin in colonic epithelium. K8 is critical for normal colon functions, as partial or full K8 loss in mice leads to a K8 dose-dependent loss of partner keratins, dysfunctional ion transport and energy metabolism, and deregulation of proliferation and differentiation. These dysfunctions in the K8-deficient mouse model contribute to a compromised barrier, inflammation and a dramatically amplified susceptibility to colorectal tumorigenesis, bearing similarities to the human multifactorial colitis syndromes. Keratins thus strongly contribute to tumor suppression and are necessary in tissue regeneration. As intestinal keratins also provide mechanical resilience and support barrier integrity, their balance is not only important for the health of our gut, but for the well-being of our entire body.

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