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# Intermediate filament proteins of digestive organs: Physiology and pathophysiology

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**Abbreviations: APL**, acquired partial lipodystrophy; **FPLD2**, Dunnigan familial partial lipodystrophy; **GFAP**, glial fibrillary acidic protein; **IBD**, inflammatory bowel disease; **IF**, intermediate filament protein; **IF-pathies**, IF-associated diseases. **K**, keratin; **MDB,** Mallory-Denk bodies; **NASH**, nonalcoholic steatohepatitis; **PTM**, post-translational modifications; **SEK**, simple epithelial keratins.

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**Abstract**

Intermediate filament proteins (IFs), such as cytoplasmic keratins in epithelial cells and vimentin in mesenchymal cells and the nuclear lamins, make up one of the 3 major cytoskeletal protein families. Whether in digestive organs or other tissues, IFs share several unique features including stress-inducible overexpression, abundance, cell-selective and differentiation-state expression, and association with >80 human diseases when mutated. While most IF mutations cause disease, mutations in simple epithelial keratins 8, 18 or 19, or in lamin A/C predispose to liver disease with or without other tissue manifestations. Keratins serve major functions including protection from apoptosis, providing cellular and subcellular mechanical integrity, protein targeting to subcellular compartments, and scaffolding and regulation of cell signaling processes. Keratins are essential for Mallory-Denk body aggregate formation that occurs in association with several liver diseases, while an alternate type of keratin and lamin aggregation occurs upon liver involvement in porphyria. IF-associated diseases have no known directed therapy, but high-throughput drug screening to identify potential therapies is an appealing ongoing approach. Despite the extensive current knowledge base, much remains to be discovered regarding IF physiology and pathophysiology in digestive and nondigestive organs.

## Overview, features and functions of intermediate filaments

Intermediate filament proteins (IFs) make up the largest family among the three major cytoskeletal protein families in mammalian cells (25,31). IFs include a large group of cytoplasmic and nuclear proteins, encoded by ~70 human genes, that are expressed cell-selectively (12,15,27). For example, keratins are preferentially expressed in epithelial cells, while vimentin is found in mesenchymal cells, glial fibrillary acidic protein (GFAP) in glial and stellate cells, desmin in myocytes (smooth, cardiac and skeletal) and lamins in the nucleus (Table 1). Of the IF gene family, keratins (K) are the largest subfamily and include 54 genes that encode keratin types I (K9-K28 and K31-K40) and II (K1-K8 and K71-K80) proteins (9,63,74). In digestive type organs, the primary keratins consist of the simple epithelial (i.e., single-layered) keratins K7/K8/K18-K20/K23 (SEK) (52). These SEKs, as other IFs, have preferential patterns of expression (Figure 1A) within the intestinal epithelium (e.g., K20 is found primarily in the more differentiated cells), in ductal versus non-ductal epithelia (e.g., K19 is expressed in biliary epithelia but not in adult hepatocytes), and in metaplastic epithelia as in Barrett's esophagus (9,51). In polarized epithelia, SEKs are located below the apical terminal web region and are anchored to the apical junction complex (31,60). All IFs are normally distributed in the cytoplasm except for the type V nuclear lamins (Table 1), but accumulating evidence indicates that, in some contexts such as cancer, IFs may also be found at low levels in the nucleus (24).

IFs, as a group, share several unique properties (Figure 1A). For example, despite the fact that keratins make up 0.2-0.5% of total cellular proteins in hepatocytes and enterocytes (87), they are stress-inducible and their protein and mRNA levels increase several fold as noted during regenerative repair in the pancreas, toxin exposure and oxidative stress in the liver, and interlukin-6 simulation in the intestine (17,75,82,87). Aside from induction of endogenous IFs

during cell stress, expression of 'new' IFs is also a hallmark of epithelial to mesenchymal transition during cancer progression in most cancers including gastrointestinal tumors (40,61). IFs include dynamic and interchangeable filamentous and soluble pools with the latter making up nearly 5% of the total K8/K18 fraction in cultured human colonic HT29 cells (52). Interchange between the soluble and insoluble IF pools is regulated by post-translational modifications (PTM) such as phosphorylation and acetylation (66) and by interaction with a growing list of IF-associated proteins (67). IFs are obligate noncovalent tetramers that are either hetero-tetramers (e.g., two type I and two type II keratin molecules) or homo-tetramers (e.g., vimentin or desmin) (12,63).

IFs also carry out several important subcellular, cellular and tissue functions that, in part, reflect their cell-selective expression (Figure 1B). One critical IF function is to provide cellular and subcellular mechanical integrity which at the cellular level is evident from the cell fragility disorders of the skin that are caused by epidermal keratin mutations (12,27,39). Similarly, hepatocytes are remarkably fragile when isolated by liver perfusion in the context of K18 mutation or absence of keratins (29,38,52). At the subcellular level, IFs play important roles in nuclear and mitochondrial shape and function (40,52,60,72) and in the targeting of proteins to the apical and other subcellular compartments and maintenance of the epithelial barrier (52,60). Another key function for IFs is to protect cells from apoptosis as has been clearly demonstrated for K8/K18 in the liver whereby keratin-null hepatocytes are markedly more susceptible to cell death as compared with wildtype cells (9,30,4352). However, the importance of keratins as anti-apoptotic proteins is context-dependent since K8-null enterocytes are paradoxically more resistant to apoptosis, compared with their wildtype counterparts, and this resistance reverts to normal after treatment of the mice with antibiotics (20). Other important functions for IFs

(Figure 1B) include their role in cell migration (40,61), modulation of protein synthesis (28), and serving as signaling scaffolds (9,52,57).

**Intermediate filaments genetic variants in digestive disease**

In contrast to most of the IF-associated diseases (IF-pathies (53)), whereby IF mutations have near-complete penetrance to cause disease, mutations in SEK predispose to, rather than precipitate disease per se (52,54,55). Among digestive organs, the liver is the primary involved organ, which is supported by multiple experimental genetic models while other organs such as the intestine, pancreas and stomach appear to be spared. The reason for this is that K8 and K18 are the only IFs expressed in adult hepatocytes while epithelia in other digestive organs express K7, K19 or K20 in addition to K8/K18 (52).

In terms of specific digestive organs, human K8 mutations appear not to be involved as either a cause or predisposition to pancreatitis or pancreatic cancer (62,80), albeit a K8 transgenic overexpression model developed spontaneous pancreatitis and acinar cell dysplasia (5). However, the latter finding is likely due to massive keratin overexpression in the studied pancreata (5,76). In addition, mice that lack K8 or K18, or overexpress mutant K18, are equally susceptible to experimental pancreatitis as compared with wild-type mice (77,78). Although genetic studies have not examined a potential role K18 or K19 in pancreatitis, it is likely that the pancreas is not a major disease target for keratin mutations given the presence of other keratins in acinar and ductal cells and the likely protective induction of Reg2 (a member of the *regenerating islet-derived stress-inducible family* of proteins) in the context of keratin mutation (88). Redundancy in keratin expression and function is also a reason why keratin variants are unlikely to play a major direct role in intestinal human disease such as inflammatory bowel disease (IBD) (73). This is despite a profound ulcerative colitis-like phenotype in mice that lack

K8 (2,21). Even though potential keratin variant association with IBD has been described (56), the number of patients in that study was relatively small and numerous genome-wide association studies (44) and other large studies (73) have not strongly implicated keratin variants in IBD.

The strongest disease association of SEK, specifically K18/K18/K19, is predisposition to acute and chronic liver disease progression in carriers of select keratin variants (9,52,54,68,74,81,89). As such, K8, K18 and K19 variants have been associated with several acute and chronic liver diseases. Many of these variants are rare (<0.1% frequency) though some are more frequent (1-5%), and it appears that these variants are silent unless the liver is challenged by an underlying viral or autoimmune chronic liver disease or acutely with a hepatotoxin such as acetaminophen (68) or isoniazid (81).

Aside from keratins, mutations in *LMNA* (the gene encoding the alternatively spliced lamin A and C proteins) result in a wide range of diseases that cause myopathy, neuropathy, premature aging or lipodystropy (4,15,85). Although lamin A/C is expressed in most differentiated tissues, its select organ disease manifestation is likely related to lamin mutation-related alteration in tissue-specific lamin-binding partners (85). Liver involvement occurs in lamin A/C mutations that cause partial lipodystrophy and metabolic syndrome including the Dunnigan familial partial lipodystrophy (FPLD2; typically heterozygous Arg482 substitutions with Trp, Gln or Leu), with hepatosteatosis that progresses to nonalcoholic steatohepatitis (NASH) in some patients (1,16,41). Liver involvement has also been described in a patient with acquired partial lipodystrophy (APL) harboring a mutation in *LMNB2* which encodes lamin B2 (13). It is probable that liver involvement was present in the first association of lamin B2 mutations in 4 of 9 patients with APL, described by Hegele et al, though liver involvement was not discussed (22).

**Intermediate filaments aggregation in the context of Mallory-Denk bodies and porphyria**

Keratins are critical for the formation of Mallory-Denk bodies (MDB); based on extensive studies carried out in genetic animal models (52,69,86). Formation of MDB aggregates occurs in the context of several liver diseases, particularly alcoholic and nonalcoholic liver disease (69,86). K8 and K18 form the major protein components of MDB, and induction of a K8>K18 ratio primarily in the context of steatohepatitis is essential for their formation (18,52,86). The significance of selective upregulation of K8 is that it serves as a substrate for transglutaminase 2, which in turn crosslinks K8 via Lys-Gln isopeptide bond formation to several other proteins (32,70). Keratin phosphorylation is also necessary for MDB formation since mice that overexpress the phospho-mutant K8 S74A (or overexpress the natural human variant G62C that leads to a conformational change which blocks kinase access to K8 S74) have a markedly decreased ability to form MDB (32). Notably, K8 G62C is a natural human variant that predisposes to liver disease in humans (52,68) and mice (19,52).

Keratins and lamins also aggregate upon exposure to protoporphyrin-IX and other porphyrins (11,42,64). Lamins A/C and B1 appear to be more sensitive than keratins to protoporphyrin-IX mediated aggregation in livers of mice harboring a ferrochelatase mutation, or mice treated with the porphyrinogenic and MDB-inducing compound, 3,5-diethoxycarbonyl-1,4-dihydrocollidine (64,86). Most of the biochemically detectable aggregation is light-induced and occurs upon exposure of cell or tissue lysates to light in the presence of porphyrins (11,42). However, porphyrins can also cause end-stage liver disease in patients (65), clearly independent of light, and in this context there is also evidence of porphyrin-mediated light-independent protein aggregation (42). It remains to be determined whether porphyrins cause keratin and lamin aggregation (and likely other IFs such as GFAP in stellate cells) in liver by forming covalent adducts (which have not be described to date for any porphyrin) or via other mechanisms such as

free radical mediated protein alterations. The mode of porphyrin-mediated protein aggregation, the physiological consequences, and the involved proteins are different to what occurs in MDB (e.g., the nuclear changes that are associated with porphyria). It remains to be determined whether some of the porphyria manifestations, as found in the skin and internal organs (e.g., abdominal pain), are directly related to the observed porphyrin-induced protein aggregation.

**Intermediate filaments as biomarkers of cell death and as differentiation and cancer markers in digestive disorders**

Most IFs, including type I but not type II keratins, are substrates for caspases during apoptosis-associated injury in experimental models or humans with release of apoptotic fragments into cell culture media or the blood stream (30,43,52). One likely consequence of caspase-mediated keratin cleavage during apoptosis is to allow the keratin filaments to reorganize with subsequent generation of apoptotic bodies. This is based on the inability of K8/K18 filaments to reorganize, and shunting of hepatocytes to necrosis rather than apoptosis, upon Fas ligand stimulation of transgenic mice (or hepatocytes ex vivo) that express caspase cleavage-resistant K18 (due to K18 mutation at its two caspase cut sites) (83).

SEK are also released upon necrosis and cell death in epithelial tumors (30). Of note, type II keratins (e.g., K7 and K8) are resistant to digestion by caspases while type I keratins (e.g., K18 and K19) generate readily detectable fragments due to the abundance of keratins (30). In acute and chronic liver injury alone, there have been more than 130 studies that assessed the association of keratin fragments with liver disease (30). Aside from pathological conditions, there is also extensive apoptotic activity and caspase digestion of K18 during normal fetal development (37). Although there are several limitations in the current assays that measure various serum K8/K18/K19 levels, including the lack of an assay that measures the product

formed after cleavage at the second K18 caspase cut site (also conserved in K19), the utility of such assays remains a useful adjunct for the assessment of liver injury and possibly other digestive organ damage (6,30).

Even within simple epithelia, the somewhat selective expression of SEK renders them useful markers particularly for defining normal differentiation states as well as the cell origin of cancers and their metastases (since tumors generally maintain their original keratin expression patterns) (47). For example, K20 is expressed upon terminal differentiation and is found most prominently in small intestine villus-lining and colon surface-lining epithelial cells (aside from being found in other cell types such as gastric faveolar cells and the urothelium) (47). In addition, specific keratin staining using well-established antibodies is routinely done world-wide as a histopathologic diagnostic aide (45,47), including the use in assessing circulating tumor cells in patients with colorectal cancer (84). Furthermore, keratins may play a modulatory role in blunting the development of colon cancer though this possibility remains to be explored further. For example, K8-null mice, which develop spontaneous colitis and hyperproliferation (21), are predisposed to developing colonic tumors (as compared with their K8+/+ and K8+/- counterparts) upon treatment with azoxymethane or breeding with the Apc(Min/+) mice (46).

**Potential therapeutic approaches**

Although major advances have been made in linking mutations in IF genes to >80 IF-pathies, there remains no specific directed current treatment for any IF-pathy, and in contrast with microfilaments and microtubules there are no known small molecules that interact directly with IFs. For digestive organ-associated IF-pathies, treatment can be envisioned and justified for severe acute liver failure whereby K8/K18 variants pose mainly a predisposition that manifests in a full-blown setting after a second-hit such as exposure to a liver toxin. However, one

potential challenge is that nearly 25 K8/K18 variants have been identified to date (52,68), and it is possible that individualized targeted therapy will be needed depending on the pathogenesis of the mutation. In this context, the use of RNA therapeutics that specifically target the mutant allele is an attractive possibility (35). Our approach has been to focus on identifying compounds that can normalize the effect of K18 filament disrupting mutations as a proof-of-concept and a model keratin that could potentially extend to other IFs (71). Using this approach, the pan-kinase inhibitor PKC412 was identified as a compound that normalizes mutant K18 R90C disrupted filaments in cultured cells and in transgenic mice (33). This occurs by dephosphorylation of the keratin binding protein non-muscle myosin-IIA which in turn promotes its binding to K8/K18 and stabilizing the keratin filaments (33). One advantage of this unbiased drug screening approach is that it has the potential to illuminate novel keratin and other IF signaling pathways or interactions that would otherwise be difficult to elucidate (33,71).

One of the major challenges in identifying therapies for IF-pathies is that what works in cell culture and in animal models has not been effective in humans as noted in more than one drug trial for the Hutchinson-Gilford progeria syndrome that is caused by lamin A/C mutation (8). However, current efforts aimed at unbiased drug screening (36), or targeting aberrant signaling pathways that are triggered by lamin A/C mutation (48) are promising and may be of benefit in FPLD2 or APL that also involve the liver.

**Closing perspective**

Tremendous gains have been made in the IF field since the first link of IFs to human disease was made for epidermal keratins in 1991 (12). The IF field remains exciting and wide open for many new important discoveries, and hopefully new comers will join the field. Areas that remain to be explored span the spectrum of fundamental biophysical, structural and biochemical

understanding of IFs and their PTM (7,23,66) to disease association, diagnosis and therapy (8,33,35,36,48,71). For example, determining the crystal structure of IFs has been very challenging due to their inherent structure, and only subdomains of some of the IFs have been crystallized (7,34). In terms of the quest for therapies for the IF-pathies and potential compound screening, the use of organism models that have a rapid generation time and genetic tools, such as Drosophila (3), is likely to be beneficial.

From the disease perspective, there are many gaps that remain to be filled including: (i) defining the disease association for several 'orphan' IFs that have not been directly linked as a cause or predisposition to human disease (e.g. for SEK: K7, K20, K23); (ii) determining whether known or novel keratin variants that have been associated with a human disease (e.g., in K8, K18, K4, K13) are involved as genetic modifiers of additional diseases. This includes gastric or intestinal disorders for K8/K18 and esophageal disorders for K4/K13 (among other keratins). In the liver, potential association of K8/K18 variants with steatohepatitis or with liver transplant failure is deserving of study. In the esophagus, K4 and K13 are the major keratins in the suprabasal layer (though they are also found in the oral mucosa) (14). K4/K13 mutation is only known as the cause of white sponge nevus syndrome (58,59) yet the phenotype of K4-null mice in the esophagus is more extensive (49) and includes nuclear atypia. Therefore, much more remains to be learned regarding keratin function and disease association in the esophagus; (iii) determining the pathophysiologic role of keratins that have been minimally studied such as the recent characterization of K23 during mouse and human biliary cell activation (17), (iv) defining the digestive disease association of non-epithelial IFs such as vimentin, GFAP and neurofilaments. For example, it is possible that variants of non-epithelial IFs could modulate gastrointestinal disorders in a non-canonical fashion. For example, GFAP is the major IF of glial

cells and it is increasingly evident that glial cells are likely to play pleiotropic roles in the enteric nervous system (50). Similarly, the functional roles of vimentin in epithelial tumor metastasis and epithelial-mesenchymal transition remain poorly understood (26,61).

Other relevant areas of future study include a better understanding of the nature and utility of IF proteolytic fragments in different digestive disorders (30), the potential role of IFs in regulating the microbiome or regulation by the microbiome (20), the pathophysiologic of IFs as stress proteins (75) which includes enhancing our limited understanding of their transcriptional control, and the subcellular and organellar functions of IFs (72,79). Overall, there is plenty of room to expand on what is known regarding IF properties, functions and disease association (Figure 1 and Table 1). IFs remain exciting to study in both digestive and non-digestive organ contexts, with many to-be-made discoveries.

## Table 1: Intermediate Filament Proteins

| Type | Proteins | Expression cell compartment | Examples of associated diseases* |
|---|---|---|---|
| I | K9-K28; K31-40 (hair and nails) | Epithelial tissues for K1-K28 (obligate type I-II heteropolymers) | EBS (K5/K14); predisposition to acute or chronic liver disease (K8/K18/K19) |
| II | K1-K8; K71-K86 (hair and nails) | | |
| III | Vimentin | Mesenchymal cells, including lens | Cataracts |
| | GFAP | Glial cells | Alexander disease |
| | Desmin | Muscle cells | Desmin-related myopathy |
| | Syncoilin | Muscle cells | Unknown |
| | Peripherin | Peripheral neurons | Amyotrophic later sclerosis |
| IV | Neurofilaments (light, medium, heavy: NF-L, NF-M, NF-H) | CNS neurons | CMT type 2, amyotrophic lateral sclerosis (predisposition) |
| | α-internexin | CNS neurons | Unknown |
| | Nestin | Stem and neuroepithelial cells | Unknown |
| | Synemin | Muscle cells | Unknown |
| V | Lamins | Nuclei | FPLD (lamin A/C); APL (lamin B2) |
| VI | Bfsp1 (Filensin), Bfsp2 (CP49) | Eye lens | Juvenile-onset cataracts |

*For some of the IFs, only a few examples of associated diseases are listed (e.g., many other keratinopathies and laminopathies are not included but none of none are known to involve digestive organs). Abbreviations: APL, acquired partial lipdystrophy; *Bfsp, beaded filament structural protein; CMT, Charcot-Marie Tooth; CNS, central nervous system; EBS, epidermolysis bullosa simplex; FPLD2, Dunnigan familial partial lipodystrophy; K, keratin.

## Figure Legends

**Figure 1. Properties and functions of intermediate filament proteins**. The schematic lists well-defined properties (A) and functions (B) for IFs. Some examples of the IF-associated diseases are listed in Table 1.

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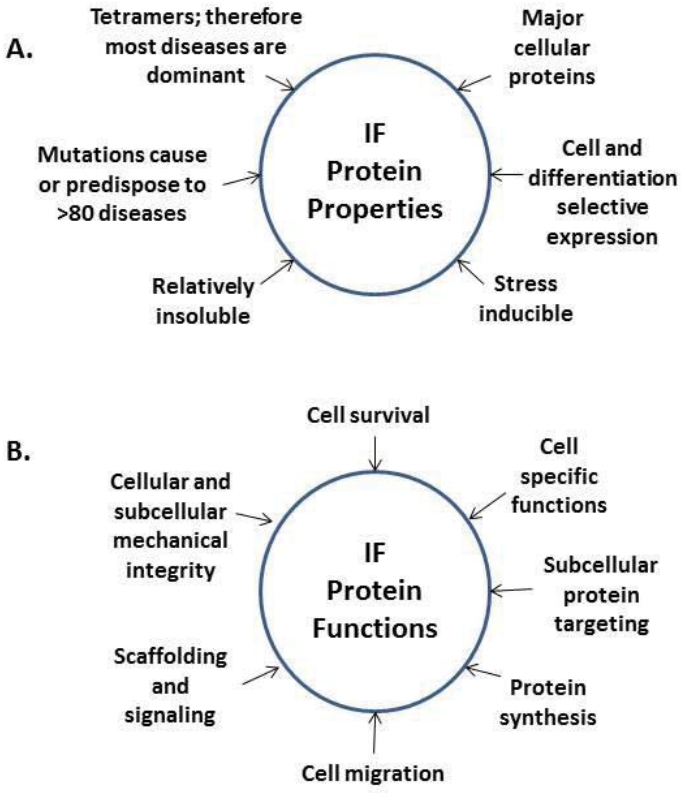
**Figure 1**


A.
Tetramers; therefore most diseases are dominant
Major cellular proteins
IF Protein Properties
Mutations cause or predispose to >80 diseases
Cell and differentiation selective expression
Relatively insoluble
Stress inducible
B.
Cell survival
Cell specific functions
Cellular and subcellular mechanical integrity
IF Protein Functions
Subcellular protein targeting
Scaffolding and signaling
Protein synthesis
Cell migration