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Networking galore: Intermediate filaments and cell migration

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Abstract

Intermediate filaments (IFs) are assembled from a diverse group of evolutionarily conserved proteins and are specified in a tissue-, cell type-, and context-dependent fashion in the body. IFs are involved in multiple cellular processes that are crucial for the maintenance of cell and tissue integrity and the response and adaptation to various stresses, as conveyed by the broad array of crippling clinical disorders caused by inherited mutations in IF coding sequences. Accordingly, the expression, assembly and organization of IFs are tightly regulated. Migration is a fitting example of a cell-based phenomenon in which IFs participate as both effectors and regulators. With a particular focus on vimentin and keratin, we here review how the contributions of IFs to the cell's mechanical properties, to cytoarchitecture and adhesion, and to regulatory pathways collectively exert a significant impact on cell migration.

Ten nanometer wide intermediate filaments (IFs), first described as such in muscle by Holtzer and colleagues [1], are assembled from the most diverse and heterogeneous group of proteins among intracellular cytoskeletal fibers. There are ~70 genes that code for IF-forming proteins in the human genome, with 54 of them coding for keratin proteins that occur primarily in epithelia [2,3]. IFs can be partitioned into six major subtypes based on gene substructure or sequence homology within their signature central rod domain (Figure 1A). All IF proteins share the property of self-assembly into ~10-nm wide filaments (Figure 1B), which they do as obligatory or facultative heteropolymers, along with a defining tripartite domain structure consisting of a central α-helical rod domain featuring long range, coiled-coil forming heptad repeats that is flanked by variable end domains located at their N- and C-termini (Figure 1C). Collectively, IF proteins exhibit pronounced heterogeneity – for instance, their molecular mass ranges from 40 kDa (type I keratin 19) to 240 kDa (type IV nestin) – though individually their primary structure is evolutionarily well-conserved. IF systems are present across multi-cellular eukaryotes [4]. The evidence in hand suggests that they appeared as nuclear proteins related to the current-day lamins in lower eukaryotes such

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as *Dictyostelium* [5]. The presence of the IF-like crescentin in *Caulobacter crescenti* [6] raises the intriguing prospect that IFs might have been born earlier, in prokaryotes.

Another remarkable signature feature of the IF superfamily of genes and proteins is the tissue type-, differentiation program-, and context-dependent nature of their regulation (Figure 1A). Consistent with this, the list of functions fulfilled by IFs in their natural biological setting is growing rapidly - by now all major facets of cell biology, including cell motility, have been linked to IFs and their associated elements (see [3,7,8]). Given their status as abundant fibrous elements within cells, IFs can impact cellular migration from mechanical and cytoarchitectural perspectives. IFs also impact migration from a regulatory perspective, owing to their ability to interact with and regulate various cellular effectors including signaling molecules [3].

As should become clear from this text, there are IF proteins, e.g., vimentin (Figure 2B), that consistently stimulate cell migration and invasion independent of the setting while others, e.g., various keratins, exert a more variable, nuanced, and at first sight complicated, impact on these processes. Beyond the type of IF protein, additional determinants such as the level at which it is expressed, its associated partners, intracellular organization and covalent modifications (e.g., phosphorylation) are acting in concert to define the overall impact on intricate processes such as cell migration. Further, cellular and biological context is crucially important. The expression “networking galore” (cf. title for this review) is meant to convey the recurring notion that the nature and impact of various IFs during migration in normal as well as disease settings reflects their pervasive integration, in a context-dependent manner, within the broader fabric of the cell.

Basic attributes of IFs relevant to their properties and function in vivo

As is the case for F-actin and microtubules, IFs depend on an array of partner proteins for their assembly, organization, function, and regulation. In particular, plakin family proteins are “cytoskeletal organizers” that anchor IFs, microtubules and actin at several strategic locations within cells [9]. Beyond their signature plakin domain, plakin family members tend to be large and exhibit a modular substructure that enables them to act as versatile organizers of the cytoskeleton [10]. Plakin proteins mediate IF attachment to the cytoplasmic “plaque” domain in cell-cell desmosome adhesions and cell-matrix hemidesmosome adhesions, to other elements of the cytoskeleton (F-actin, microtubules), and to the surface of the nucleus [9–11].

IF proteins are regulated by several types of post-translational modifications including phosphorylation, O-glycosylation, ubiquitination, sumoylation, and acetylation [12–14]. Such modifications are site-specific within the IF protein backbone, are typically reversible (and often dynamic), and regulate virtually all aspects of their assembly, organization, properties, and function [3,15,16]. In combination, associated proteins and post-translational modifications help define the polymerization status and intracellular organization of IFs in their natural setting. Actively migrating, polarized cells tend to have their IF system reorganized around the nucleus or at their rear, trailing end, in natural settings [17–19] and under conditions of mutant IF protein expression [20].

Interplay between intermediate filaments, adhesion, and other cytoskeletal elements

Desmosomes are comprised of transmembrane cadherins, armadillo proteins such as plakoglobin and plakophilins, and plakin proteins such as desmoplakin that link desmosomal plaques to IFs intracellularly [11] (Figure 2A). Desmosomes maintain tissue integrity under

mechanical stress [11] beginning at an early stage during mouse embryogenesis [21]. Potent pro-migratory cues such as epidermal growth factor (EGF) regulate the assembly and functional state of desmosomes (and hemidesmosomes) and IF network architecture [22–25]. Stimulation of cell migration is generally coupled to weaker desmosome-dependent cell-cell adhesion [26]. Indeed, enhanced desmosome turnover and their reduced colocalization with keratin have been observed in migrating oral squamous cell carcinoma cells [27].

Cell migration is also a function of dynamic interactions between ECM components and the cell cortex (Figure 2A). The transmembrane, adhesion-mediating entity in hemidesmosomes is the α6β4 integrin heterodimer, which provides a cell surface receptor for extracellular laminin [28]. Intracellularly, integrin linkage to IFs is mediated by plakin proteins including the bullous pemphigoid antigens 1 and 2 (BPAG1, BPAG2), and plectin (Figure 2A; [10]). In the complete absence of keratin, hemidesmosome components are scattered in skin keratinocytes which, paradoxically, adhere faster to the ECM and show increased migration relative to wild-type [29]. Re-expression of the K5–K14 keratin pair alone (typical of progenitor basal keratinocytes) in such keratin-free skin keratinocytes reverses this phenotype, even when at a sub-physiological level. By comparison keratinocytes null for BPAG1 show a normal density of hemidesmosomes at the cell-matrix interface, but lack a cytoplasmic plaque and attachment to keratin IFs, and exhibit a delay in their ability to cover a wound site in skin *in situ* [30]. The knockdown of actinin-4, an actin-binding protein, results in a loss of directionality during the migration of individual keratinocytes, correlating with a mislocalization of α6β4 integrin and BPAG1e (Figure 2A) and defects in cell polarity and lamellipodial dynamics [31]. The p90 ribosomal protein S6 kinase (RSK) has been implicated in hemidesmosome remodeling [32,33] and in the regulation of the wound-inducible keratin 17 [34], raising the issue of its influence in complete keratin-null and/or actinin-4 knockdown keratinocytes. Besides, Bordeleau *et al.* showed that the knockdown of keratin 8 (K8) in cultured hepatoma cells impaired cell migration in a scratch-wound assay [35], decreased cell surface area upon spreading, altered Rho-dependent actin fiber organization, and decreased local stiffness at focal adhesions, reflecting an interplay between K8/K18 IFs and Rho-mediated actin dynamics occurring through plectin, RACK1 and Src [36] (see below).

LINC (linker of nucleoskeleton and cytoskeleton) is a protein complex present at the nuclear membrane that participates in anchoring the nuclear lamina to cytoskeletal proteins on the cytoplasmic side [37] (Figure 2A). Nesprin-3, a component of LINC, also associates with plectin [38]. Disruption of LINC via expression of mutated nesprin impairs intracellular force transmission, alters the organization of F-actin and vimentin IFs, and causes impaired migration and polarization in mouse embryonic fibroblasts [39]. Similarly, depletion of Nesprin-3 in human aortic epithelial cells alters the organization of vimentin IFs and impairs cell migration [40]. On a related front, depletion of the major IFs in astrocytes (nestin, vimentin, glial fibrillary acidic protein) alters the position and rotation of the nucleus during astrocyte migration [41] and impairs their migration [42]. Such findings build upon the observation that cell migration entails dynamic changes in the position and shape of the nucleus [43], and that IFs contribute to nuclear architecture in skin keratinocytes [3] and migrating cells [43].

A keratin-containing multi-protein partnership may be regulating the pace of keratinocyte migration

The significance of the partnerships between IFs and their associated proteins is adeptly conveyed by the converging migration phenotypes exhibited by several genetic null mutants in mouse skin keratinocytes. Genetic loss of epiplakin, a plakin family member, in mouse

results in enhanced skin keratinocyte migration [44] alongside loss of keratin IF bundling post-wounding [45]. Enhanced migration also occurs in mouse keratinocytes genetically null for plectin [46], plakoglobin [47] plakophilin [47], and keratin 6 (K6a/K6b) [48,49]. Further, the loss of either K6a/K6b, plectin or plakoglobin occurs alongside Src family kinase activation and altered F-actin reorganization [44,46,50]. A follow-up effort on the plectin deficiency phenotype suggested that IFs may indirectly regulate the organization and stability of microtubules via an interaction with the plectin1c isoform, specifically, and an associated impact on focal adhesion dynamics and directional migration of keratinocytes [51].

The powerful Src kinase is known to regulate leading edge protrusion through Rac and Cdc42 signaling, and stimulate focal adhesion dynamics and formation of invadopodia; besides, Src can also directly induce epithelial-to-mesenchymal transitions (EMT; see Box 1). In the study involving K6a/K6b null keratinocytes, Src was shown to directly interact with keratin IFs in a K6-dependent fashion via a novel, non-phosphotyrosine-mediated contact involving Src's SH2 domain, which dampens its enzymatic activity [49]. Also, Src's partitioning to detergent-resistant membranes, a locale where it is transiently inactive, is mitigated in K6a/K6b null keratinocytes [49]. Whether such findings also apply to epiplakin, plakophilin, plakoglobin and/or plectin null keratinocytes is an issue now worth examining, as is the relationship of these findings to those of Bordeleau et al. [36], discussed above. Much remains to be learned about this keratin-containing multi-protein partnership and the mechanism(s) and effector(s) through which it so adeptly regulates keratinocyte migration. The apparent paradox between the wound-inducible character of K6 and its negative influence of "pure" cell migration (i.e., as seen in the "favorable" setting of *ex vivo* culture) has also been observed for several other cytoskeletal proteins. One must now seek to understand how various elements contribute to determine the optimal speed and mode of cellular migration in a given biological context (see [19]).

Box 1

Intermediate filaments, epithelial-to-mesenchymal transition (EMT), and tumor growth, invasiveness, and metastasis

Invasion of the proximal connective tissue stroma by cancer cells is a critical initial step in cancer metastasis. Epithelial cancer cell invasion is typically accompanied by an epithelial-to-mesenchymal transition, or EMT, so-called because epithelial cells typically lose their polarity and other defining characteristics (e.g., E-cadherin, keratin expression) as they adopt a fibroblast-like morphology (including vimentin expression) and aggressive migratory properties. One of the key differences between the epithelial and mesenchymal phenotypes lies in the tight cell-cell and cell-matrix contacts made by epithelial cells compared to the loosened contacts of mesenchymal cells (see [106], [107] for excellent reviews on this topic). The process of EMT is increasingly appreciated as an important mechanism to account for the enhanced motility and invasiveness of epithelial tumor cells [71,108,109].

As epithelial cells undergo EMT, their IF system switches from being keratin-dominated to vimentin-dominated, which is characteristic of mesenchymal cells. Many cancer cell lines exhibit both a keratin-based and a vimentin-based IF network that show distinct intracellular organization and regulation [62,110]. The pioneering work of Mary Hendrix and colleagues shed an "early light" on the significant relationship between IFs and cell migration, and its relation to the potential for metastasis. These studies, whether cell-culture-based [111,112] or using xenograft assays *in vivo* [113], revealed the pro-migratory influence of vimentin, the impressive power of an interplay between two types of IFs (namely vimentin and keratins 8/18) towards invasiveness, and the important role

of focal adhesions and integrins, in particular. As such, this work is still inspirational today.

Keratin 6 and its type I partner K16 are often upregulated in various types of carcinomas, providing clinically useful diagnostic markers [52]. K6's impact on keratinocyte migration (and possibly the K6-Src interaction) could help explain a series of intriguing clinical correlations [53]. For instance, loss of K6 expression correlates with an aggressive behavior for endometrial carcinomas [54], while reduced K6 expression coupled with re-emergence of K8/K18 expression correlates with the acquisition of malignancy in mouse skin subjected to chemical carcinogenesis [55]. These correlations suggest that the functional significance of inducing or modifying K6 (and possibly K16, plakoglobin, plectin, etc.) may be part of a natural strategy to counter dedifferentiation- and malignancy-promoting signaling and cellular processes (e.g. EMT) [49]. This said, other findings remind us that the link between keratins and cancer (see [52]) is not so simple. Higher levels of K16, for example, correlate with a poorer survival among breast cancer patients with metastatic relapses [56], while higher levels of keratins 5, 6 and 17 have been linked to a worse prognosis in breast cancer [57–59]. Again here, whether a given keratin or a conglomerate of IF proteins and binding partners promote or mitigate cell migration and/or tumor cell properties likely is determined by the overall "context" – e.g., associated proteins, post-translational modifications, and the biological setting.

Intermediate filaments, cellular mechanics, and migration

Cells develop a polarized cytoarchitecture as they initiate cell migration, such that their front and rear become different in their molecular components and functional properties [60,61]. As a cell senses relevant environmental cues, signaling events, actin polymerization, and myosin motor function each become spatially regulated so as to generate membrane protrusions at the leading edge and retractive forces at the trailing edge. Mechanical signals participate in the establishment of polarized cell protrusions and directional migration and, as expected, there is evidence that IFs impact cell migration from the standpoint of cellular mechanics [29,62,63].

Mechanotransduction involving IFs also plays a role in epithelial cell attachment to the extracellular matrix (ECM). Zhang *et al.* [64] uncovered a mechanotransduction pathway in *C. elegans* that involves hemidesmosome-like elements comprising IFs. They observed that muscle contraction mechanically alters the epidermis and activates p21-activated kinase (PAK). PAK, in turn, phosphorylates IF proteins, an event that promotes hemidesmosome biogenesis. Therefore, hemidesmosomes act as mechanosensors which, when subject to tension, trigger intracellular signaling processes that promote epithelial morphogenesis. Cell-cell junctions participate in the integration of local traction forces to generate long-range gradients of intra- and inter-cellular tension during collective cell migration [65]. While studying *Xenopus* gastrulation, Weber *et al.* [19] found that application of a punctual mechanical force on single *Xenopus* mesendoderm cells (via magnetic tweezers and cadherin-coated beads) induces polarized protrusions at the opposite end of the force (and the cell) and persistent directional cell migration. Such localized tension ("tugging") induces, in a plakoglobin-dependent fashion, a redistribution of the keratin IFs at the cell's rear end (see Figure 2A for a summary of these findings). These striking events centered on keratin and plakoglobin are required for force-induced, polarized "group" cell protrusions and normal mesendoderm polarity and organization *in vivo*.

Vimentin as a powerful enhancer of cell migration in normal tissues and tumor settings

Vimentin is a fascinating type III IF protein that is prominently expressed throughout embryogenesis but becomes largely restricted to mesenchymal cell types in the adult setting, including fibroblasts, bone marrow-derived blood cell lineages, and endothelial cells [66,67]. Vimentin can re-emerge in the adult setting, as it is strongly upregulated following injury to various tissues (e.g., muscle, central nervous system, various connective tissues) and during EMT (see Box 1). Vimentin exerts pleiotropic and context-dependent roles in cells [68] and, in particular, has a marked impact on cell migration in several physiologically normal settings [69]. For example, vimentin is required for lymphocyte adherence to and migration through an endothelium [70], fibroblast or breast cancer single cell motility [71], and *in vitro* wound closure of alveolar epithelial cells [72] (see Table 1 for a summary of cell migration phenotypes arising from IF manipulations).

Vimentin also occurs at unusually high levels in many types of epithelial cancers (e.g., [68]). Vimentin expression is in fact required for the invasive phenotypes of prostate cancer cells [73,74], soft tissue sarcoma cells, and breast cancer cells, in *in vitro* assays [75]. Blocking vimentin expression in a squamous carcinoma cell model not only decreases motility [76] but also promotes a more epithelial phenotype, as manifested by the upregulation of K13, K14, and K15 [77] and change in cell shape [71]. Conversely, vimentin overexpression has been shown to enhance prostate cancer cell invasion [78] and invadopodia elongation [79].

Numerous studies incorporating a more mechanistic focus hint that vimentin and the process of cellular migration mutually regulate one another. The tumor suppressor adenomatous polyposis coli (APC), which is frequently mutated or lost in cancer (e.g., colorectal), directly binds to and regulates vimentin organization [80]. In migrating astrocytes, APC is required for vimentin IF alignment with the microtubule network [80]. A C-terminal APC truncation mutant binds to and disorganizes vimentin, but not keratin IFs, when expressed in human SW480 colon cancer cells. Loss of APC in cancer cells that have undergone EMT may thus alter vimentin IF organization and impact motility and invasiveness (see Figure 2B for a summary). In cultured breast epithelial cells, overexpression of oncogenic H-Ras-V12G or the transcription factor Slug, each of which promote cell migration and EMT [81], induces vimentin expression. In turn, vimentin expression is required for H-Ras-V12G- and Slug-induced migration and expression of receptor tyrosine kinase Axl, while suppressing epithelial markers such as K6 [81]. Overexpression of Axl rescues the slower migration phenotype of a breast cancer cell line expressing vimentin siRNA, suggesting that vimentin acts in part through Axl.

An RNAi screen aimed at identifying regulators of vimentin expression yielded the surprising finding that the mitochondrial enzyme MTHFD2 (methylenetetrahydrofolate dehydrogenase 2) is required for vimentin expression and network organization [82]. Similar to vimentin itself, the siRNA-mediated knockdown of MTHFD impairs breast cancer cell migration and ECM invasiveness, suggesting their interdependence in this context.

Vimentin expression is also regulated by miRNAs. Overexpressing mir-138, which is downregulated in several tumors, results in decreased vimentin expression as well as decreased cell migration and invasion in renal cell carcinoma cell lines [83] (Figure 2B). Similarly, mir-30a represses vimentin expression, cell migration and invasion, in breast cancer cell lines [84]. Since some tumor cells exhibit decreased expression of mir-138 and mir-30a [83,84], these findings may help explain how vimentin expression becomes upregulated in EMT and cancer.

The complex relationship between keratins 8/18 and epithelial cell migration

The type II keratin 8 has also been implicated in cell migration and tumor metastasis [35,85]. Like vimentin, K8 is quite broadly expressed during development but becomes restricted to simple epithelial lineages (e.g., liver, gut, kidney, lungs) in the adult setting [86]. Further, K8 expression is induced or elevated in many tumor settings (including breast, lung, and pancreatic cancers) and tumor-derived cell lines [86]. Unlike the case for vimentin, however, the impact of K8 on tumor cell migration and invasion tends to be inhibitory. This said, the pioneering work of Mary Hendrix and colleagues two decades ago showed that the balance between vimentin and K8/K18 expression is a key determinant of the migratory properties and invasiveness of various types of tumor cells *ex vivo* and *in vivo* (Box 1).

In an elegant study published in 2003, Beil *et al.* [87] showed that treatment of pancreatic cancer Panc-1 cells with sphingosylphosphorylcholine (SPC), a bioactive lipid, induces keratin phosphorylation, promotes a striking reorganization of keratins IFs to the perinuclear region, decreases cellular elasticity, and robustly stimulates cell migration (Figure 2B). A pair of recent offerings provided additional details relevant to this paradigm. Park *et al.* [88] showed that SPC treatment also induces the expression of transglutaminase-2 expression in Panc-1 cells, which precedes JNK kinase activation and phosphorylation at K8 Ser 431. Busch *et al.* [89] reported that SPC activates ERK kinase upstream of keratin IF reorganization, and induces phosphorylation of K8 and K18 at Ser 431 and Ser 52, respectively, in pancreatic and gastric cancer cells. An open issue, still, is whether these events contribute to the “mechanical softening” of the cytoplasm in SPC-treated Panc-1 cells [87], an event that likely contributes to their more motile behavior.

There is plenty of additional reports intimating that, directly or indirectly, the expression and/or site-specific phosphorylation of K8 (and its partner K18) impacts the migratory properties and invasiveness of various types of cancer cells. An inhibitory influence for K8 towards cell migration is suggested by studies in which pancreatic cancer cells [89] and a poorly invasive subclone of MDA-MB-468 breast cancer cells was subjected to K8 knockdown [90], a highly invasive subclone of MDA-MB-435 breast cancer cells was made to overexpress K8 [90], and when KLE endometrial cancer cells and HepG2 hepatocellular cancer cells were subjected to K8/K18 silencing [91]. Other studies related the loss of K8 phosphorylation at either Ser 73 or Ser 431 to increase migration and/or metastatic potential for oral squamous cell carcinoma cells [92] and colorectal cancer cells [93]. The opposite outcome, i.e., K8-dependent stimulation of cell migration, was inferred from the impaired collective migration of hepatoma cells following K8 silencing [35]. Finally, the silencing of the desmosomal plaque protein plakophilin 3 stimulates the migration and metastasis of human colon carcinoma cells [94], and a recent follow-up study suggests that this is likely a function of increases in the levels of K8 protein and the phosphatase PRL-3, along with K8 de-phosphorylation [95].

Keratin-dependent activation of Akt signaling may also play a role during tumorigenesis. Lactotransferrin (LTF) has anti-tumor activity and is downregulated in cancer [96]. Interaction with LTF blocks K18’s binding to 14-3-3σ, and suppresses K18-mediated Akt activation and its associated impact on tumor cell proliferation and invasion [97] (Figure 2B). Of note, others have reported that K17 interacts with 14-3-3σ and impact the Akt-mTOR signaling axis [98], while vimentin interacts with and becomes activated by Akt to promote cancer cell invasion [75]. Also of note though not yet related to migration *per se*, O-linked N-acetylglucosamine modification of K18 also promotes Akt activity to protect the liver from injury [99].

In addition to vimentin and keratin, increased expression of nestin, a class IV IF protein and a marker of stem/progenitor cells [100], occurs in multiple types of tumors [101,102]. Nestin regulates the migration and metastatic properties, but not the growth, of prostate cancer cells [103] and pancreatic cancer cells [104]. Due to its link to stem/progenitor cells, it now seems timely to investigate whether nestin expression can be used to identify cancer stem cells [105].

Concluding remarks

Recent advances added significantly to our current understanding of the complex role of IFs during cell migration. IFs impact migration in part because they are intrinsic determinants of cellular micromechanical properties, and also because they contribute to the regulation of several pathways and effectors that are intimately involved in this physiologically important activity. In the end, the notable impact of IFs during cell migration in normal and disease settings reflects their pervasive integration, in a context-dependent manner, within the broader fabric of the cell.

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A

Subgrouping	Proteins	Cell type specificity
Type I Type II	Keratins	Soft complex epithelia (skin, oral mucosa, etc.) Soft simple epithelia (liver, gut, kidney, etc.) Hard epithelia (hair, nail, oral papillae)
Type III	Vimentin, Desmin GFAP, Peripherin syncoilin	Various (fibroblasts, leukocytes, endothelium muscle, astrocytes, glia, peripheral nerves)
Type IV	NF-L, NF-M, NF-H a-internexin synemin, nestin	CNS & neurons CNS & neurons Muscle, neural stem cells
Type V	Lamins A, B & C	Nucleus
Orphan	Filensin, Phakinin	Lens

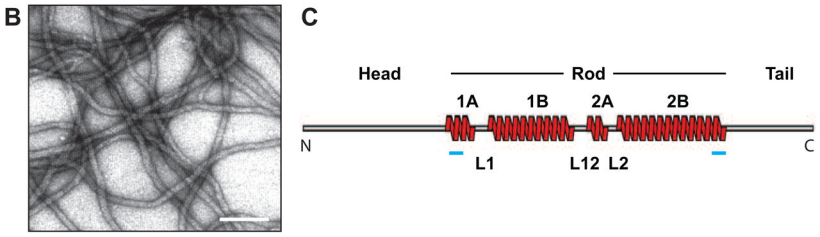

Figure 1.

Introduction to intermediate filaments (IFs). A) Classification of IF genes and proteins by type, according to gene substructure and sequence homology, and cell type-specificity of their distribution in the body (note: the latter list is partial). B) Visualization of assembled 10-nm wide IFs reconstituted from purified recombinant proteins (the type II K5 and type I K14; human) by negative staining and transmission electron microscopy. Bar equals 100 nm. C) Schematic representation of the common tripartite domain structure shared by all IF proteins. A central domain, comprised of heptad repeat-containing α-helical coils 1A, 1B, 2A, and 2B and separated by non-heptad repeat-containing linkers L1, L12 and L2, is flanked by “head” and “tail” domains of variable length and primary structure at the N- and C-termini, respectively. The boundaries of the rod domain (see blue bars) are highly conserved in primary structure among IF proteins.

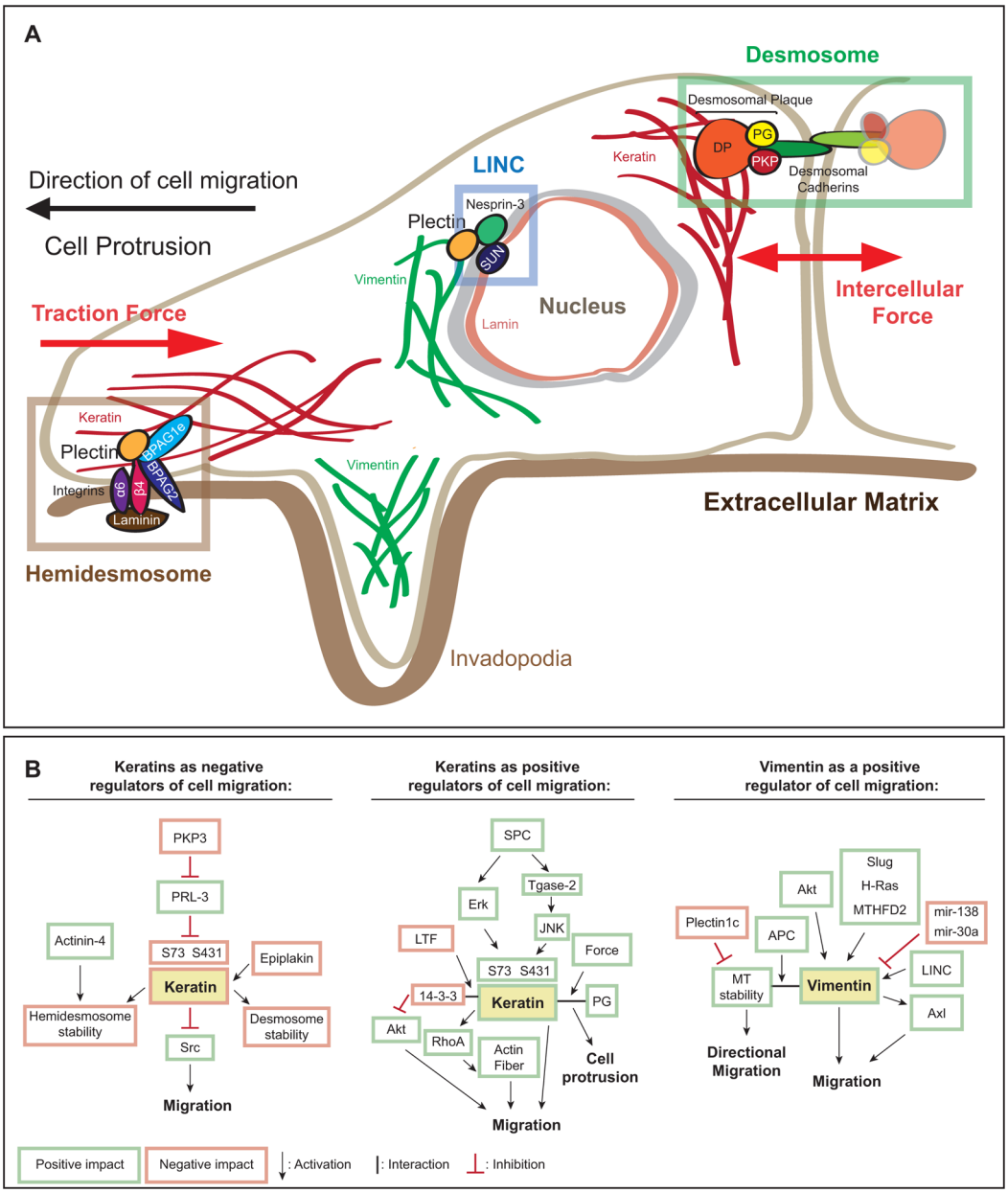

Figure 2.

Function and regulation of intermediate filaments in cell migration. A) Schematic representation of a polarized migrating cell highlighting the subcellular distribution of vimentin and keratin IFs and their associated elements. See text for explanation. B) Summary of key interactions involved in specifying keratins as negative (left) or positive (center) regulators of cell migration, and in specifying vimentin as a positive regulator of migration and key contributor to epithelial-to-mesenchymal transition (EMT). See main text for details. Abbreviations are as follows: α6: α6 Integrin; APC: Adenomatous Polyposis Coli; β4: β4 Integrin; BPAG1e: Bullous Pemphigoid Antigen 1e; BPAG2: Bullous Pemphigoid Antigen 2; DP: Desmoplakin; Erk: Extracellular Signal-related Kinase; JNK: c-Jun N-terminal Kinase; LINC: Linker of Nucleoskeleton and Cytoskeleton; LTF: Lactotransferrin; MT: Microtubule; PG: Plakoglobin; MTHFD2: Methylenetetrahydrofolate dehydrogenase 2; PKP: Plakophilin; PRL-3: Phosphatase of Regenerating Liver 3; S73 &

S431: Serine 73 and Serine 431 of K8; SPC: Sphingosylphosphorylcholine; SUN: Sad1-Unc84; Tgase-2: Transglutaminase-2.

Table 1

Impact of expression and site-specific phosphorylation of select intermediate filament proteins during cell migration. This list inventories the expression and site-specific phosphorylation events on either vimentin or select keratin proteins that have been implicated in the regulation of cell migration. Information about cell type, genetic manipulation, type of migration assay used, and source is given.

IF protein examined	Cell type, Species (all in ex vivo culture)	Assay/Migratory stimulus	Genetic Alteration	Observed Effect	Reference
Keratin (all)	Skin keratinocytes (mouse)	Scratch wound assay	Global Krt Null	increased migration	[29]
K5/K14	Skin keratinocytes (mouse)	Scratch wound assay	Expression in Krt Null	decreased migration	[29]
K8	hepatoma cells (rat)	Scratch wound assay	K8 shRNA	decreased migration	[36]
	breast cancer cells (MDA MB 435; human)	Scratch wound assay	K8 overexpression	decreased migration	[90]
	breast cancer cells (MDA MB 468; human)	Scratch wound assay	K8 shRNA	increased migration	[90]
	colorectal carcinoma cell line (HCT116; human)	Scratch wound assay	K8 shRNA in PKP3 shRNA	decreased migration (compared to PKP3 shRNA)	[95]
K8/K18	endometrial cancer cell line (KLE; human)	Scratch wound assay	shRNA	increased migration	[91]
K8/K18	hepatocellular cancer cell line (HepG2; human)	Scratch wound assay	shRNA	increased migration	[91]
K18	pancreatic cancer cells (Panc-1; human)	random migration	K18 siRNA	increased migration	[89]
K6	Skin keratinocytes (mouse)	ex vivo explant assay	K6a/K6b Null	increased migration	[49]
Vimentin	Lymphocytes	In vivo homing assay	Vimentin Null	decreased migration	[70]
	Embryonic Fibroblast (mouse)	random migration	Vimentin Null	decreased migration	[71]
	breast cancer cell line (MCF-7; human)	random migration	Vimentin overexpression	Increased migration	[71]
	breast cancer cell line (MDA-MB-231; human)	Scratch wound assay	Vimentin siRNA	decreased migration	[76]
	colon cancer (SW480; human)	Scratch wound assay	Vimentin siRNA	decreased migration	[76]
	bronchoalveolar carcinoma cell line (H358; human)	Scratch wound assay	Vimentin overexpression	Increased migration	[72]
	Alveolar epithelial cells (rat)	Scratch wound assay	Vimentin shRNA	decreased migration	[72]
	breast cancer cell line (MDA-MB-231; human)	Scratch wound assay	Vimentin siRNA	decreased migration	[81]
	mammary epithelial cell line (MCF-10A; human)	Scratch wound assay	Vimentin siRNA	decreased migration	[81]
	MCF-10A H-Ras-V12G (human)	Scratch wound assay	Vimentin siRNA	decreased migration	[81]
	prostate cancer cell line (PC-3; human)	Scratch wound assay	Vimentin siRNA	decreased migration	[78]
	prostate cancer cell line (PC-3; human)	transwell migration assay	Vimentin siRNA	decreased migration	[78]
	prostate cancer cell line (PC-3; human)	transwell invasion assay	Vimentin siRNA	decreased invasion	[78]
	colorectal carcinoma cell line (HCT116; human)	Chemoinvasion assay	Vimentin siRNA	short invadopodia	[79]
	breast cancer cell line (MDA-MB-231; human)	Chemoinvasion assay	Vimentin siRNA	short invadopodia	[79]
	breast cancer cell line (MDA-MB-231; human)	Chemoinvasion assay	DN vimentin mutant (1–138)	short invadopodia	[79]

IF protein examined	Cell type, Species (all in ex vivo culture)	Assay/Migratory stimulus	Genetic Alteration	Observed Effect	Reference
	Head/neck squamous cell carcinoma cell line (HN12; human)	transwell migration assay	Vimentin siRNA	decreased migration	[77]
	Head/neck squamous cell carcinoma cell line (HN12; human)	transwell invasion assay	Vimentin siRNA	decreased invasion	[77]
	prostate epithelial cancer cell lines (1E8-H; human)	transwell migration assay	Vimentin siRNA	decreased migration	[74]
	prostate epithelial cancer cell lines (1E8-H; human)	transwell invasion assay	Vimentin siRNA	decreased invasion	[74]
	prostate epithelial cancer cell lines (2B4-L; human)	transwell migration assay	Vimentin overexpression	increased migration	[74]
	prostate epithelial cancer cell lines (2B4-L; human)	transwell invasion assay	Vimentin overexpression	increased invasion	[74]
	prostate cancer cell line (LNCaP; human)	transwell migration assay	Vimentin overexpression	No change	[73]
	prostate cancer cell line (LNCaP; human)	transwell invasion assay	Vimentin overexpression	No change	[73]
	prostate cancer cell line (LNCaP-CL1 subline)	transwell migration assay	Vimentin siRNA	No change	[73]
	prostate cancer cell line (LNCaP-CL1 subline)	transwell invasion assay	Vimentin siRNA	decreased invasion	[73]
Vimentin and GFAP	Astrocytes (mouse)	random migration	Vimentin and GFAP null	decreased migration	[42]
Nestin, Vimentin and GFAP	Astrocytes (rat)	Scratch wound assay	siRNA	alters the positioning and rotation of the nucleus	[41]
Phosphorylation event	**Cell type, Species (all in ex vivo culture)**	**Assay/Migratory stimuli**	**Genetic alteration**	**Observed Effect**	**Reference**
K8 S73	oral squamous cell carcinoma cell line (human)	Scratch wound assay	Overexpression (S73A)	Increased migration (compared to OE WT)	[92]
K8 S431	oral squamous cell carcinoma cell line (human)	Scratch wound assay	Overexpression (S431A)	Increased migration (compared to OE WT)	[92]
K8 S431	Panc-1 pancreatic cancer cells (Panc-1) (human)	random migration	Overexpression (S431E)	Increased migration (compared to OE WT)	[89]
	Panc-1 pancreatic cancer cells (Panc-1) (human)	random migration	Overexpression (S431A)	no change in migration (compared to OE WT)	[89]
Decreased K8 phosphorylation	Panc-1 pancreatic cancer cells (Panc-1) (human)	random migration - SPC stimulation	MEKK inhibitor (U0126)	decreased migration	[89]
	Panc-1 pancreatic cancer cells (Panc-1) (human)	Boyden chamber assay - SPC stimulation	MEKK inhibitor (U0126)	decreased migration	[89]