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Vimentin as a potential molecular target in cancer therapy Or Vimentin, an overview and its potential as a molecular target for cancer therapy

Arun Satelli and Shulin Li

MD Anderson Cancer Center Department of Pediatrics, Unit 853 1515 Holcombe Blvd. Houston, TX 77030

Abstract

Vimentin, a major constituent of the intermediate filament (IF) family of proteins, is ubiquitously expressed in normal mesenchymal cells and is known to maintain cellular integrity and provide resistance against stress. Increased vimentin expression has been reported in various epithelial cancers including prostate cancer, gastrointestinal tumors, CNS tumors, breast cancer, malignant melanoma, lung cancer and other types of cancers. Vimentin's over-expression in cancer correlates well with increased tumor growth, invasion and poor prognosis; however, the role of vimentin in cancer progression remains obscure. In the recent years, vimentin has gained much importance as a marker for epithelial-mesenchymal transition (EMT). Although EMT is associated with a number of tumorigenic events, the role of vimentin in the underlying events mediating these processes remains unknown. Though majority of the literature findings indicate a future significance of vimentin as a biomarker for different cancers with clinical relevance, more research in to the molecular aspects will be crucial to particularly evaluate the function of vimentin in the process of tumorigenesis. By virtue of its over-expression in a large number of cancers and its role in mediating various tumorigenic events, vimentin serves as an attractive target for cancer therapy. Further, research directed toward elucidating the role of vimentin in various signaling pathways would open up new approaches for the development of promising therapeutic agents. This review summarizes the expression and functions of vimentin in cancers and also suggests some directions toward future cancer therapy utilizing vimentin as a potential target.

Introduction

The microfilaments, IFs and microtubules comprise the three major nonmuscle cell cytoskeletal proteins. IF family of proteins are encoded by a large gene family of ~70 members in humans, the mouse and other mammals (1). There are six major classes of IFs and are believed to be restricted to certain cell types (2). These include, type I and II (acidic and basic keratins, mainly found in the epithelial cells), type III - vimentin (mesenchymal cells) and desmin (muscle cells), type IV – neurofilaments (neurons), type V- lamins (nucleus of cells) and type VI- nestin (embryonic neurons). Further, there are the IF associated proteins (IFAPs) that organize intermediate filaments in bundles and networks, which include proteins like plectin, ankyrin, desmoplakin, and filaggrin (3). These IFAPs are known to coordinate the interactions between IFs and other cytoskeletal elements and organelles. Together, IFs and IFAPs serve as organizers of cytoplasmic space within cells

Correspondence to: Dr. Shulin Li MD Anderson Cancer Center UTMD Graduate School of Biomedical Sciences Department of Pediatrics, Unit 853 1515 Holcombe Blvd. Houston, TX77030 Phone: 713-563-9608 Fax: 713-563-5407 sli4@mdanderson.org.

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and cells that make up the tissue architecture, thereby providing stability and strength to various organs (3).

Vimentin, a 57 kDa protein, is one of the most widely expressed and highly conserved proteins of the type III IF protein family. During murine development, vimentin expression commences on embryonic day 8.5 and its expression is predominant in the primitive streak stage (4), while in adults vimentin expression is limited to connective tissue mesenchymal cells, in CNS and in muscle (5). Vimentin is expressed in a wide range of cell types, including pancreatic precursor cells, sertoli cells, neuronal precursor cells, trophoblastic giant cells, fibroblasts, endothelial cells lining blood vessels, renal tubular cells, macrophages, neutrophils, mesangial cells, leukocytes and renal stromal cells (6–11). Vimentin has gained much importance as a canonical marker of EMT (reviewed in (12)), a cellular re-programming process in which the epithelial cells acquire a mesenchymal phenotype that renders the cells to dramatically alter their shape and exhibit increased motility. This EMT is characterized by the expression of vimentin IFs in epithelial cells, which normally express only keratin IFs. Accordingly, during the reverse process of EMT, known as mesenchymal-epithelial transition (MET), the cells start acquiring epithelial phenotype and show a decreased vimentin expression with lower motility rates (13). Increased vimentin expression has been reported in various tumor cell lines and tissues including prostate cancer, breast cancer, endometrial cancer, CNS tumors, malignant melanoma and gastrointestinal tumors including pancreatic, colorectal and hepatic cancers; further details are discussed in later sections of this review.

Although vimentin is considered to maintain the structural processes of the cell and mediate many other fascinating functions *in vitro* (reviewed in (14)), knockout models of mice lacking vimentin showed virtually normal phenotypes and did not reveal any apparent defects (15). This observation suggested that vimentin is not critical for the survival of the mice under normal physiological conditions. However, later studies involving a much more detailed evaluation, showed that the vimentin (–/–) mice exhibit impaired wound healing in both embryonic and adult stages due to the weak and severely disabled fibroblasts that were not in a capacity to migrate (16, 17), died due to end-stage renal failure in a pathological situation involving the reduction of renal mass when compared to their wild-type littermates (18), showed decreased flow-induced dilation during arterial remodeling, suggesting that vimentin modulates arterial structural responses to altered blood flow (19) and finally, vimentin deficient lymphocytes showed a decreased homing capacity to lymph nodes and spleen (20). Interestingly, vimentin (–/–) embryonic stem cell-derived tumors showed similar pattern of composition of teratocarcinomas as induced by wild-type embryonic stem cells, suggesting that vimentin is not essential for efficient tumor growth and differentiation *in vivo* (21), while several *in vitro* results suggest a tumor promoter role of vimentin in cancer (14). The discrepancy in these results can be attributed to the fact that vimentin is differentially expressed in different cell types and it might play a tissue-specific function or there might be a redundancy in the function among IF proteins. Thus, the use of vimentin knockout mice can provide valuable information on the roles of vimentin and also contribute towards bridging the gap between *in vitro* results and clinical data. Further, understanding the expression pattern and function of this protein in a wide variety of cancerous tissues can provide new diagnostic, prognostic and therapeutic approaches to tackle this deadly disease.

Structure and Regulation of vimentin

Vimentin is a 53 kDa polypeptide comprised of 466 amino acids, with a highly conserved α -helical “rod” domain that is flanked by non- α -helical N- and C-terminal end domains, termed “head (77-residue)” and “tail (61-residue)”, respectively (22). Together, these molecules associate in parallel and in-register to form a coiled-coil, which forms the basic

structural building block for the entire IF family of proteins (23). Vimentin is also known to form homopolymers and heteropolymers (associates with other type III or IV IFs), a common feature shared among the members of the IF family, which is attributed to the presence of a coiled-coiled alpha-helical domain that helps in the formation of highly stable polymers, the stability of which in turn is controlled by the phosphorylation status of the integral proteins (24). The head domains of a vimentin dimer are known to form a symmetric structure and three sites have been identified that indicate an increase in the distance between the head regions upon phosphorylation, a post-translational modification that regulates vimentin assembly/disassembly (25–27).

Vimentin serves as an excellent substrate for a number of kinases *in vitro* and multiple phosphorylation sites on vimentin have been identified (28–32). Phosphorylation of vimentin is associated with functional consequences including the regulation of IF structure and several signaling pathways (26). For example, protein kinase A (PKA) phosphorylated vimentin primarily on sites S38 and S72 that leads to decreased filament formation *in vivo*; however, site directed mutagenesis of these sites showed no significant effect on filament assembly, indicating that phosphorylation primarily regulates disassembly of vimentin IFs (26). p21-activated kinase (PAK) was shown to phosphorylate vimentin at several sites and was involved in the regulation of vimentin structural reorganization (33). Aurora B Kinase, a key regulator involved in the mitotic processes was shown to phosphorylate vimentin and regulate the vimentin filament segregation during cytokinetic process (34). Further, protein phosphatase 2A (PP2A) was shown to associate with vimentin and prevent its phosphorylation, suggesting that PP2A plays a key role in the regulation of interphase IF dynamics (35). Vimentin has also shown to be a target for other types of post-translational modifications including citrullination, sumoylation and O-GlcNAc modification. Citrullination is a post-translational modification in which the arginine residues are enzymatically deiminated to citrulline by peptidylarginine deiminase (36). Vimentin was shown to be citrullinated in macrophages undergoing apoptosis, and antibodies to this citrullinated vimentin are generated in the event of improper disposal of apoptotic material (37). O-GlcNAcylation of glial vimentin is suggested to prevent hyperphosphorylation of vimentin, thus retaining its ability to maintain a rigid structure and provide a scaffold for neuronal migration (38). Vimentin was sumoylated at site 354 in the nucleus upon stimulation with protein inhibitor of activated STAT3 (PIAS3) and this modification is suggested to regulate the structure and motility of glioblastoma multiforme cells (39). Taken together, these studies suggest an important role of post-translational modifications in the regulation of vimentin's function. Furthermore, these modifications are cell- and tissue-specific in nature. However, additional post-translational modifications of vimentin may also remain to be identified, which can open up new areas that are essential in understanding its functions both *in vitro* and *in vivo*.

Vimentin is encoded by a single-copy gene located on chromosome 10p13. Initially, vimentin promoter was shown to be composed of three different elements that regulate its expression (40). Later, several *cis*-elements and associated factors were identified within the human vimentin promoter, suggesting that vimentin gene is subjected to complex control. These include a TATA box, eight putative GC-boxes (40), NF- κ B binding site (41), AP-1 binding site (42), PEA3 binding site (43), Sp/XKLF binding site (44) and ZBP-89 binding site (44, 45). Furthermore, vimentin expression was shown to be transactivated by β -catenin/TCF, binding to the putative site 468 bp upstream of the transcription initiation site of vimentin promoter and thus increasing the tumor cell invasive potential (46). It has been shown that NF- κ B, a key protein regulating the immune and inflammatory process, also plays an important role in regulating EMT process (47) and its inhibition in the mesenchymal cells reversed the EMT process (48), suggesting the importance of NF- κ B in both activation and maintenance of EMT. Since vimentin is over expressed during EMT

process, and NF- κ B being one of the transcription factors binding to vimentin promoter, it would be tempting to speculate that this over expression of vimentin is a result of activated NF- κ B in cancer cells. Also, TGF β 1 response element was found within the activated protein complex-1 region of the vimentin promoter and was involved in regulation of vimentin expression in myoblasts and myotubes (49).

Vimentin gene was shown to be a target of epigenetic modifications. Vimentin gene is frequently methylated in advanced colorectal carcinomas and was suggested to serve as a diagnostic marker in the detection and monitoring for colorectal carcinoma using serum and stool samples (50). Further, upon treatment with 5-Aza-Deoxycytidine, a methylation inhibitor, vimentin mRNA expression was induced by several folds in different colon cancer cell lines (51), indicating that DNA demethylation is sufficient to activate vimentin transcription in colon cancer cells. Also, it has been shown that a transcription factor ZBP-89 is known to recruit histone deacetylase 1 (HDAC1) to the vimentin promoter, which leads to a decrease in vimentin expression (52). This effect was abrogated in the presence of trichostatin A, a HDAC1 inhibitor, indicating that vimentin gene is vulnerable to HDACs and contributes to one of the possible mechanism of vimentin silencing. From these studies it is evident that there are a multitude of endogenous transcriptional activators or repressors that are regulating the expression of vimentin in a given cell type and these factors thus regulate the EMT-associated events that are taking place during the cell migration process. A rational drug design to inhibit vimentin expression requires deeper insights into vimentin gene regulation and therefore further investigation can be promising in identifying newer drug targets.

Subcellular distribution and export of vimentin to cell surface

Vimentin being a cytoskeletal structural protein, is expected to be restricted to the cytosolic portion of the cells; however, vimentin has been shown to be nuclear as well as an extracellular protein. Traub et al. (53) predicted the possibility that intermediate filament (IF) proteins not only participate in the cytosolic functions but also mediate certain DNA- and RNA- mediated events in the nucleus, however, this functionality of IFs was in question because they lacked the nuclear localization sequences, which allow them to enter the nucleus. Later, a novel mechanism of vimentin entry into the nucleus by single-stranded DNAs via a piggyback mechanism was suggested (54). At the nuclear envelope, 6.6-kD tail region of vimentin was shown to interact with lamin B and was suggested to provide a continuous set of contacts between the plasma membrane skeleton and the karyoskeleton of eukaryotic cells (55). Recently, it has been shown that vimentin localizes in the nucleus of neuroblastoma cells and regulates p21Waf1 expression; however the principal events involved in this regulation still remain unclear (56). This report sheds light on the ability of vimentin to act as a regulator of transcription, suggesting that there are other protein yet to be identified that are under the control of nuclear vimentin. At present there is a scarce in knowledge about the role of vimentin in the nucleus and much more investigation is required in this area.

As stated above that vimentin is also an extracellular protein, it would be interesting to note that vimentin lacks a signal sequence for secretion; however, Perides et al. (57) have suggested that the positively charged amino terminal of vimentin, rich in hydroxylated and hydrophobic amino acid residues, can serve in directing it toward membranes and assist in binding of the protein to the lipid bilayer. Although the mechanism of this transport remains unclear, there have been several other reports of vimentin transport to the cell surface. Vimentin detected on the cell surface of cardiomyocytes and vascular smooth muscle cells, accounted for nearly $7.58 \pm 13.66\%$ and $13.53 \pm 4.33\%$ of the total biotinylated cell surface proteins (58). Moreover, extracellular staining showed punctuate like appearance,

confirming that only a fraction of the vimentin is expressed on the cell surface. In another study, it was shown that vimentin interacts with $\beta 3$ -integrins and plectin which together regulate the organization and distribution of vimentin in several different cell types (59). Further, the authors also suggested that a possible role for $\beta 3$ -integrin-mediated recruitment of vimentin to cell surface is to regulate the adhesion strength of the cells binding to the substrate. Interestingly, neutrophils undergoing spontaneous apoptosis were shown to express vimentin and lamin B₁ on the cell surface (60), which again opens up a possibility for these cells to participate in the development of corresponding autoantibodies in the serum, a condition frequently associated with several inflammatory disorders (61). Mor-Vaknin et al (62), have shown that vimentin undergoes phosphorylation in activated monocyte-derived macrophages, which leads to its expression on the cell surface and is responsible in killing bacteria and in oxidative metabolite production. The authors reported an increased association of vimentin with the endoplasmic reticulum and the Golgi, suggesting that vimentin is secreted into the extracellular milieu. This secretion of vimentin was blocked in the presence of the Golgi blocker monensin and the glycosylation blocker tunicamycin, both of which disrupt early to intermediate events involved in the conventional secretory pathway that includes vesicular trafficking through the endoplasmic reticulum-Golgi compartments leading to budding of secretory vesicles from the *trans*-Golgi network and exocytosis. Further, the inflammatory cytokine interleukin-10 blocked the secretion of vimentin through PKC inhibition, whereas the pro-inflammatory cytokine tumor necrosis factor- α , triggered secretion of vimentin (62). In another study, using mass spectrometry analysis it was discovered that vimentin secreted from the surface of on *Mycobacterium tuberculosis*-infected monocytes acts as a putative ligand for NKp46 receptors on natural killer (NK) cells (63). This binding contributes to the recognition of infected monocytes by NK cells. Further, it was suggested that this binding was highly specific to the secreted vimentin and not the intracellular vimentin. Interestingly, neutralizing the surface vimentin resulted in only 50% inhibition of the NK cytolytic activity, indicating that vimentin is perhaps acting as a cross-linking protein between the natural ligand and NKp46 receptor (63). Although NKp46 receptors are highly specific to NK cells, their expression has been reported in several other cell types including cancer cells (64), suggesting a possibility that vimentin binding to the cell surface receptors of specific cells elicits specific functions, which are yet to be investigated.

Huet et al. (65) have reported SC5, a monoclonal antibody that specifically detects the secreted vimentin at the surface of viable Sézary cells or of activated normal T lymphocytes. Further, the authors suggested the presence of anti-vimentin autoantibodies in the serum of Sézary patients, which can serve as an excellent marker for diagnosis or prognosis. Recently, utilizing proteomic analysis on serum samples from hepatocellular carcinoma (HCC) patients, it was shown that vimentin is heavily secreted by small HCCs (66). It was also shown that although few of the HCC cells did not express endogenous vimentin, they secreted vimentin. However, neither the mechanism of secretion nor the function of secreted vimentin is known. It could be possible that secreted vimentin is bound on the surface of cancer cells and attracts the natural killer cells that harbor the NKp46 receptors to the tumor site, where they get suppressed by different mechanisms (67, 68) thereby creating a tumor immune escape environment that enhances tumor progression. Taken together, these results imply that vimentin transport to cell surface is tightly controlled during development and that several factors, including cytokines can modulate vimentin's extracellular transport. It is interesting to note that although majority of the epithelial cancers do express vimentin during EMT, the extracellular location of vimentin yet remains to be investigated and, could serve as a possible target for therapeutic intervention.

Vimentin in cell signaling

Vimentin IFs are found in the cytoplasm of mesenchymal cells, where it functions to maintain the cyto-architecture and tissue integrity (4). Vimentin is known to interact with a large number of proteins and participates in various cellular functions (few of the interactors are summarized in Table 1). Further, vimentin is also involved in a number of other processes that involve formation of complexes with several cell signaling molecules and other adaptor proteins. For example, vimentin was shown to interact with phosphorylated Erk (pErk), a MAP kinase and protect it from dephosphorylation (69). Using biochemical and molecular modeling approaches, it was observed that pErk binding was localized to the second coil-coiled domain of vimentin and this binding was calcium dependent. From these observations it was suggested that vimentin is stabilizing pErk by protecting it from dephosphorylation by calcium-dependent steric hindrance thereby enabling long distance transport of phosphorylated Erk within the cell (69). AKT1 kinase was shown to bind phosphorylated vimentin and protect it from caspase-induced proteolysis that leads to increased cell motility and invasion of soft-tissue sarcoma cells (70). 14-3-3 proteins participate in a multitude of cell signaling and cell cycle processes. Phosphorylated vimentin was shown to interact with 14-3-3 protein and prevent the assembly of Raf-14-3-3 and other such complexes, thereby suggesting that vimentin regulates 14-3-3 complexes and controls various intracellular signaling and cell cycle control pathways by modifying 14-3-3 availability (71). Scrib, a protein involved in cell migration, is protected from proteasomal degradation upon interaction with vimentin, suggesting a possibility that vimentin up-regulation during EMT leads to stabilization of Scrib to promote directed cell migration to increase the invasive capacity of cells (72). Vimentin acts as a break on differentiation in immature osteoblasts by interacting with activating transcription factor 4 (ATF4) (73). Vimentin was shown to function as a regulator of Axl and that it enhances cell migration by inducing Axl (74). Further, Slug-and Ras-induced EMT changes were shown to be dependent on the up-regulation of vimentin (74). From these studies it is evident that vimentin not only acts as a scaffolding protein but also mediates several signaling pathways and cellular processes. Also, it would be interesting to find out other functions of vimentin in the nucleus with possible roles in mediating cell cycle processes. Furthermore, extracellular vimentin could also mediate several signaling processes by binding to specific receptors that are yet to be investigated. Elucidating the molecular mechanisms involved in the EMT process can provide deeper insights into the role of vimentin in perturbing different signaling pathways by targeting specific proteins.

Vimentin in Cancer

Evaluating the expression pattern of vimentin in normal and cancer tissues can be of great value in tumor diagnosis and prognosis. In this section, we summarize the observations of vimentin's expression profile and functions in different cancers that are reported by various researchers.

Prostate cancer

In prostate cancer, vimentin expression was mainly detected in the poorly differentiated cancers and bone metastases and was nearly undetectable in well differentiated tumors or in moderately differentiated tumors (75, 76). Further, vimentin expression was associated with motile prostate cancer cell lines (76) and its down-regulation in PC-3 cells led to a significant decrease in tumor cell motility and invasive activity (75). Vimentin was over-expressed in prostate cancer cell line CL1 and after experimentally abrogating the expression of vimentin, there was a significant decrease in the invasiveness of the tumor cell line (77). Interestingly, there was no change in the invasiveness of LNCaP cells after forced expression of vimentin. The authors speculated a possibility that vimentin's expression

contributes to the development of an invasive phenotype in conjunction with other yet to be discovered proteins or at a later stage of the cancer development. In another study, vimentin was shown to be over expressed in highly metastatic human prostate epithelial cancer cell line PC-3M-1E8 and its role in modulating the invasiveness was attributed for its ability to regulate E-Cadherin/ β -catenin complex via C-src regulation (78). Several other studies also supported the view that vimentin is over expressed in prostate cancer and contributes to their invasive and metastatic potentials (79, 80).

Gastrointestinal tract cancers

Gastrointestinal tract cancers include cancers of the stomach, small intestine, colon, rectum, liver and pancreas. In gastric cancers, vimentin expression was more associated with the invasive phenotype of gastric carcinoma and was suggested to play an important role in the metastasis of gastric carcinomas and serve as a prognostic marker in the detection of gastric cancers (81, 82). Analysis of esophageal squamous cell carcinoma samples from patients showed that vimentin expressing cells exhibit significantly higher incidence of lymph node metastasis (83). In HCC, vimentin expression was mainly associated with the metastatic HCC (84); however, in small HCC's, vimentin was detectable in the serum samples (66). In contrast, in another study it was observed that over-expression of vimentin in HCC cells decreased their proliferative and invasive capabilities *in vitro* (85), however the mechanisms underlying these effects remain unclear at present. In colorectal cancer, vimentin gene methylation has gained much attention recently and is suggested to occur frequently in advanced colorectal cancers (50). This phenomenon can be harnessed to develop high sensitivity techniques to detect methylation of vimentin gene in clinical samples including serum and stool (51, 86, 87). It would be interesting to know whether vimentin gene methylation can serve as a biomarker to predict the early onset or recurrence of colorectal cancer. Vimentin over-expression in colorectal cancers is mainly associated with the stromal component and is restricted to the stromal fibroblasts, endothelial cells lining the microvessels and tumor infiltrating lymphocytes (88, 89). However, over-expression of vimentin was also detected in colorectal cancer cells, which correlated well with increased migration and invasive potentials which reduced upon vimentin knock-down using vimentin-specific siRNA (90). It was also suggested that 70% knock-down of vimentin was sufficient to impair invasion and migration capacities of carcinoma cells. Proteomic expression analysis of colorectal cancer samples from patients by two-dimensional gel electrophoresis showed a differential regulation of vimentin expression when compared to surrounding normal tissue (91).

Vimentin expression in pancreatic duct-like cells starts from E12.5 in rodent embryos and reaches peak levels soon after birth most likely due to simultaneous up-regulation of TGF- β protein during this period, which was shown to up-regulate vimentin (49, 92). In pancreatic cancers, there was a 3-fold increase in vimentin expression when compared to other tumors; however, a more specific antigenic isoform of vimentin was found at 5–10 fold higher levels and autoantibodies generated against this isoform were suggested to have a utility for the early diagnosis of pancreatic cancer (93). Transient knockdown of Methyl-CpG binding domain protein 1 (MBD1), a suppressor of gene transcription led to down-regulation of vimentin mRNA levels drastically, suggesting a possibility that MBD1 mediates the expression of vimentin in pancreatic cancer (94). Also, TGF- β was shown to be over expressed in pancreatic cancers and regulate the expression of vimentin in Panc-1 cells (95). Further, vimentin was shown to increase the invasive potential of pancreatic cancer cells, which was reduced in the presence of vimentin-specific siRNA (96).

Breast Cancer

Vimentin expression was shown to be elevated in several aggressive breast cancer cell lines (46) and this over expression was very well correlated with increased migration and invasion of breast cancer cells (46, 97). Furthermore, the non-invasive MCF-7 cells upon vimentin over expression exhibited increased motility and invasiveness and these characteristics were down-regulated by vimentin antisense oligos in MDA-MB-231 cells, which constitutively express vimentin (97). It is interesting to note that vimentin was over-expressed in MCF-10A cells at the edge of the wound and provided the cells with enhanced cell migration potential which was dependent on EGF (98). Histological examination of human breast carcinoma samples showed that vimentin expression is predominantly found in high-grade ductal carcinomas with low estrogen receptor levels (99). Several other studies have reported over expression of vimentin in breast cancer cell lines and tissues (reviewed in (100)). Recent studies have reported that vimentin plays a major role in the EMT process of breast cancers and its knock-down resulted in a decrease in genes linked with breast cancer invasion and the basal-like phenotype including *Axl*, *ITGB4* and *PLAU* with a subsequent increase in the genes abundant in normal mammary epithelium including *RAB25* and *EHF* (74). Furthermore, vimentin was shown to play a key role in the regulation of *Axl* and also Slug- and Ras-induced migration in breast cancer cells (74).

Malignant Melanoma

Recently, utilizing proteomic analysis on a wide array of melanoma samples, Li et al (101), showed that over expression of vimentin in primary tumors not only serves as a diagnostic marker, but also act as a predictor of hematogenous metastasis. The authors also suggested a possibility of using vimentin expression as a predictor of clinical outcome, thereby providing individual treatment strategies for melanoma patients. Several other reports also address the relation between over expression of vimentin and its association with metastases and increased invasive potential of melanoma cells (102–105).

CNS tumors

In normal human brain, moderate to strong vimentin expression was mainly found in the ependymal cells, choroid plexus, meningeal cells and some subpial cells, while weak expression was found in endothelial cells (106). Vimentin expression in glioma cells was shown to be dependent on the cellular density and chemo/radio treatment and was predominantly expressed in low-density cell cultures (107). Further, in glioblastoma cells, galectin-1 was shown to regulate PKC ϵ and vimentin mediated integrin trafficking that are essential for propelling glioma malignancy (108). In meningiomas, specific phosphorylated form of vimentin expression was associated with the non-infiltrative tumors when compared to infiltrative/invasive tumors (109) and this form of vimentin was suggested to determine the migration potential of the meningiomas and to differentiate different type of meningiomas. Vimentin expression was also detected in schwannomas and neurofibromas (110).

Lung cancer

Vimentin is expressed in the bronchial epithelium of the fetus and decreases with increasing age, whereas in adult bronchial epithelium, its expression was mainly restricted to the basal and the columnar cells (111). In lung cancers, vimentin expression was detected in moderately and well differentiated adenocarcinomas and in giant cell carcinomas (112). In non-small-cell lung cancer (NSCLC), vimentin over-expression was found to be an independent prognosticator for poor survival in resected NSCLC patients (113). In another study it was observed that glycosylated vimentin was down-regulated in human lung adenocarcinomas and was suggested to represent new functional biomarkers for diagnosis

and treatment of lung cancers (114). Furthermore, vimentin was shown to be differentially expressed in lung cancer cell lines and Poly(ADP-ribose) polymerase-1 (PARP-1) was shown to bind the vimentin promoter region and induce its expression in lung cancer cells (115).

Other cancers

Vimentin over-expression was also found in other types of cancers including cervical cancer (116), clear cell renal cell carcinoma (117), certain type of lymphomas (118), papillary thyroid carcinoma (119), and endometrial carcinomas (120).

Taken together, it is evident that vimentin not only serves as a diagnostic tool in the detection of the cancer, but also plays a key role in the development and progression of cancer. Although its expression is more emphasized in the EMT process, it is equally possible that tumorigenic events including tumor cell migration and invasion are a consequence of vimentin over-expression in these cells, as most of the studies indicated a positive association of invasive phenotype to vimentin over-expression and showed a decrease in these characteristics upon knock-down of vimentin *in vitro*. Furthermore, vimentin's over-expression during metastasis (78, 83, 84, 101, 104) attracts much attention to further elucidate its role as a metastasis-promoter. However, these events are a result of fine tuning occurring in the cancer cells and vimentin might be acting as a scaffolding protein during signal transduction and promoting tumorigenic events in association with other tumor promoting oncogenes.

Drugs targets of vimentin

Vimentin over-expression in different cancer cell lines and tissues, and its association with increased cancer cell growth, invasion and migration, suggests a possibility that vimentin is in fact participating in the promotion of these tumorigenic events and serves as an excellent target for cancer therapy. Also, due to its over-expression in different cancers, vimentin can be used as a target to deliver therapeutic agents to the tumor site. Although majority of the reports in the literature show a decrease in vimentin levels using different therapeutic agents, this effect is always considered secondary and an indirect effect on vimentin expression, which is used as a marker to confirm reduction in EMT. However, there have been very few reports that show a direct inhibition of vimentin expression and its consequences.

Withaferin-A (WFA), a bioactive compound isolated from *Withania Somnifera*, was shown to bind tetrameric vimentin at a unique binding pocket site between the pair of head-to-tail α -helical dimers (121). Recently, it has been shown that WFA-induced apoptosis is significantly more pronounced in vimentin-expressing cells and vimentin knockdown in these cells abrogated this effect (122). The authors suggest a possible mechanism involving the degradation of vimentin upon binding to WFA, which then results in increased apoptosis of cancer cells. Silibinin, a flavolignan, and the major active constituent of silymarin isolated from milk thistle (*Silybum marianum*), that has shown strong chemopreventative and anticancer activities was shown to inhibit the invasion, motility and migration of prostate cancer cells ARCaP_M via down-regulation of vimentin and MMP-2 (123). These results were similar to the results reported by Singh et.al (124), which revealed anti-metastatic and anti-invasive activities of silibinin in TRAMP mice via its ability to re-express E-cadherin with a concomitant strong decrease in the levels of vimentin thereby inhibiting EMT. Salinomycin, an antibiotic, showed a drastic reduction in the levels of vimentin with a concomitant increase in E-Cadherin levels in CD133+ colorectal cancer cells (125). Although salinomycin exhibited anti-cancer properties by down regulating cell growth, migration and invasion, the mechanism underlying these events involving vimentin remains elusive. Recently, microRNAs (miRs) were shown to significantly reduce vimentin

expression in anaplastic thyroid carcinomas (ATCs) (126). Pools of miR induced MET in ATCs, which was accompanied by an increase in E-cadherin and a decrease in vimentin protein and mRNA levels. However, it is still unclear as to how these miRs inhibit the expression of vimentin and induce an epithelial phenotype with decreased invasive potential in cancer cells.

Our lab has recently identified a novel linear peptide called comprehensive carcinoma homing peptide (CHP, sequence VNTANST), which when administered in the form of CHP-IL12 (DNA fragment encoding VNTANST sequence was inserted directly before the stop codon of the p40 subunit in an IL-12 plasmid DNA) fusion gene construct, resulted in an increased accumulation of IL-12 in the tumor microenvironment and showed promising results in different cancer models (Ref). From the results reported in this paper, it is evident that this peptide possesses excellent targeting and homing properties, which makes it a useful tool to treat different cancers. From mass spectrometry analysis it was observed that one of the binding partners for CHP was vimentin. These observations indicate a distinct possibility that vimentin is expressed on the cell surface of cancer cells and gets internalized upon contact with specific ligands. The fact that lung tissue expresses vimentin but there was no CHP binding and a decrease in CHP binding upon neutralization with vimentin antibodies suggests that CHP is specifically binding to the surface-bound vimentin on cancer cells. It is equally possible that CHP upon internalization interacts with intracellular vimentin and interferes with various signaling pathways thereby affecting different cellular functions; however, this possibility is yet to be tested. This report has thus identified vimentin as an attractive target to direct the therapeutic agents to the tumor site. Currently, we are in the process of testing the effects of co-administering CHP with anti-cancer agents, as recent studies have reported a significant increase in the efficacy of anti-cancer drugs that were co-administered with tumor penetrating peptide fragments (127). In the recent years, aptamers have gained a lot of attention in the field of cancer therapeutics (128) and identification of vimentin-specific aptamers will be of great value, as aptamers are known to exhibit high binding specificity and can be chemically modified to improve their therapeutic properties. Further, vimentin specific antibodies can be used to deliver anti-cancer agents to the tumor site. Therefore, it would be of utmost importance to check the profile of vimentin expression in different cancer types with respect to its localization and different isoforms, which can then shed light on development of novel treatment options.

It would be interesting to note that majority of the targets discussed here have been tested *in vivo* and did not exhibit any significant toxicity, indicating that these therapeutic modalities are specific for vimentin expressed in cancer cells and not for the normal mesenchymal cells. A possible explanation for such an outcome could be either lower expression levels of vimentin in normal cells compared to EMT transformed cells or difference in subcellular localization patterns in these cells or expression of vimentin variant in cancer cells. Thus by utilizing vimentin as an anti-cancer target, there will be a plethora of therapeutically pertinent opportunities to overcome the current predicaments in cancer therapy.

Concluding Remarks

From all the above studies, it is evident that vimentin is a multifunctional protein and its ability to interact with a large number of proteins, makes it a potential regulator of several different physiological functions; however the true function of vimentin apart from maintaining the structural integrity of the cells is yet to be unraveled. In majority of the cancers vimentin is over-expressed and studies link its expression to the aggressiveness of the cancer. Importantly, expression of vimentin is mainly associated with metastatic phenotype and poor prognosis of the disease outcome. Interestingly, cancer related studies address the functions of intracellular vimentin, however, the role of extracellular/cell surface associated vimentin still remains unclear. Understanding the mechanism of vimentin gene

regulation and the role of extracellular/cell surface vimentin can contribute to the better understanding of cancer and control the invasiveness of the cancer cells. Though all findings indicate a future significance of vimentin as a biomarker for different malignancies with clinical relevance, more research will be necessary to particularly assess the major function of vimentin in the process of tumorigenesis. Moreover, utilizing vimentin knockout mice, valuable information on the role of vimentin in cancer can be established, which can also be used to bridge the gap between *in vitro* results and clinical data. In view of the available data, vimentin expression in cancer serves as an attractive and a promising therapeutic target and has immense potentialities in terms of novel clinical prognostic and diagnostic tools. Further, the use of vimentin specific chemical inhibitors as well as novel therapeutic agents including antibodies/peptides/aptamers/siRNA directed against vimentin in combination with other anticancer agents must be encouraged.

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Figure 1. Vimentin's role in cell signaling

In cytosol, vimentin was shown to interact with phosphorylated-Erk and protect the phosphorylation by inhibiting phosphatases which allows it to travel long distances within the cell. Also, vimentin's interaction with 14-3-3 proteins prevents the formation of 14-3-3-Raf complex and thereby regulates several cell processes by depleting the availability of free 14-3-3. Further, AKT1 was shown to phosphorylate vimentin and protect it from caspase induced proteolysis; therefore the freely available vimentin is participating in processes that lead to increased migration and invasive capacity of the cells. In the nucleus, vimentin was shown to regulate p21 expression, while the complex formed between ATF4 and vimentin prevents the active transcription by ATF4. Extracellularly, vimentin specific receptors are not yet identified, however one of the possible receptors NKp46 was identified on natural killer cells to which vimentin acts as a specific ligand. Although reports indicate that vimentin is also secreted, neither its function nor the mechanism of secretion is clear.

Figure 2. Vimentin's role in cancer

Over-expression of vimentin is frequently associated with increased migration/invasion capacity of the cells. Vimentin is mainly used as a marker for EMT in association with other known markers. Majority of the cancers over express vimentin and it is used as an indicator of poor prognosis.

Table 1

This table shows different interactors of vimentin and their possible functions.

INTERACTING PROTEIN	FUNCTION	REFERENCE
TSGA10	Influences the function of antigen presenting cells (APC)	(1)
AptA (A. phagocytophilum toxin A)	Activates mammalian Erk1/2 mitogen-activated protein kinase	(2)
Scrib	Cell migration and aggregation	(3)
Rab9	Intracellular lipid transport	(4)
Caveolin-1	Anterior polarization of caveolin-1 in transmigrating cells	(5)
Plectin	Integration of cytoplasm	(6)
IFAP-300	Lens cell differentiation	(7)
hsc70	Regulation of heat-shock genes	(8)
Filamin	Formation of cell extensions	(9)
Alpha-crystallin	Enhanced vimentin aggregation	(10)
cGMP kinase	Vimentin phosphorylation	(11)
Yes kinase	Molecular support for Yes kinase	(12)
Desmoplakin	Links IFs with desmosomes	(13)
Periplakin	Mediates cellular localization	(14)
Actin-containing structures	Vimentin filament organization	(15)
hnRNP	Viral replication	(16)
Uridine phosphorylase	Function unknown	(17)
Formiminotransferase cyclodeaminase	Integrates Golgi complex with IF cytoskeleton	(18)