

Intermediate Filaments in Breast Cancer Progression, and Potential Biomarker for Cancer Therapy: A Narrative Review

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Abstract: Intermediate filaments are one of the three components of the cytoskeletons, along with actin and microtubules. The intermediate filaments consist of extensive variations of structurally related proteins with specific expression patterns in cell types. The expression pattern alteration of intermediate filaments is frequently correlated with cancer progression, specifically with the epithelial-to-mesenchymal transition process closely related to increasing cellular migration and invasion. This review will discuss the involvement of cytoplasmic intermediate filaments, specifically vimentin, nestin, and cytokeratin (CK5/CK6, CK7, CK8/CK18, CK17, CK19, CK20, CSK1), in breast cancer progression and as prognostic or diagnostic biomarkers. The potential for drug development targeting intermediate filaments in cancer will be reviewed.

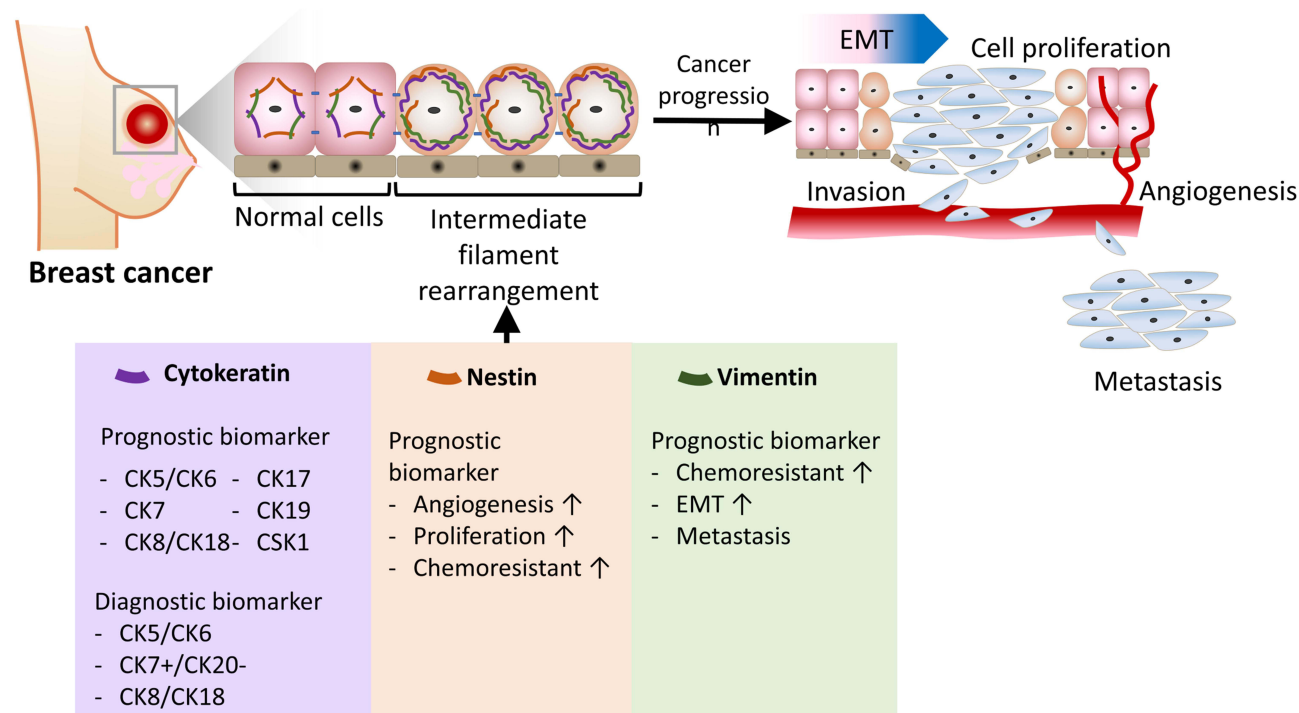
Keywords: breast malignancy, cytokeratin, EMT, intermediate filament, keratin, nestin, vimentin

Introduction

During tumor progression, the mutated cells undergo malignant transformation that induces a sequence of structural rearrangements. This cellular transformation is closely related to the Epithelial-to-Mesenchymal Transition (EMT) process that allows the cells to gain mobility and leads to the ability of the cells to migrate and invade the blood vessel, leading to cancer metastasis at a distant site. Multiple biochemical modifications happen to epithelial cells that undergo the EMT process to gain mobility and extracellular matrix (ECM) component productibility of mesenchymal cells. The biochemical mechanism includes initiation of EMT by transcription factors, expression of specific cell-surface proteins, cytoskeletal modification and rearrangement, enzyme secretion for ECM degradation, and alteration in specific microRNA expression. Thus, it leads to cancer progression and metastasis to distant sites.^{1–5}

The cytoskeleton's intermediate filament plays a prominent role in maintaining cell integrity. The rearrangement of intermediate filaments, including vimentin, nestin, and keratins, has also been reported to be involved in cancer malignancy.⁶ Over the years, various intermediate filaments have been used in scientific and clinical studies as biomarkers to assist in identifying tumor types and prognoses and determining appropriate cancer treatment.^{7–9} The exploration of intermediate filaments has been studied in various cancers, including breast cancer. This cancer is ranked second after lung cancer for the mortality-to-incidence rate, which accounts for 15%.¹⁰ Breast cancer holds the first place globally as the highest cancer incidence in both sexes (11.7%). While in the female population, the incidence rate of breast cancer reaches 24.5%, with a mortality rate of 15.5%.¹¹

Graphical Abstract



This review will summarize the roles of intermediate filaments in cancer studies and the progression of breast cancer, focusing on three types of intermediate filaments: vimentin, nestin, and keratin. We will also discuss the potential of intermediate filaments as molecular targets for cancer therapy.

Intermediate Filament in Cancer Studies

The intermediate filament comprises the largest and the most diverse cytoskeletal protein family besides actin filament and microtubules.¹² The intermediate filament is divided into six types (type I–VI). The first four types of intermediate filament (type I–IV) can be found in the cytoplasm of various cells. Type V of intermediate filament consists of lamins located in the nucleus, known as nuclear lamins. The last type of intermediate filament (type VI) can be found in the lens.⁶ The major characteristics of intermediate filaments are their rope-like structure and excellent strength, which help cells withstand mechanical stress and maintain their structural integrity. The intermediate filament is abundantly found in the cytoplasm of cells prone to mechanical shears, such as neural cells, skin epithelial cells, and mobile mesenchymal cells.¹³

In cancer studies, staging is generally determined by evaluating cancer size and its spreading to nearby tissue (T), lymph nodes (N), and distant organs or metastasis (M). Based on this TNM system, cancer progression can be classified into 5 stages (Table 1). Cancer development starts with the accumulation of abnormal changes or dysplasia in a group of cells called carcinoma in situ (CIS).¹⁴ CIS has the potential to develop into cancer later and form a primary tumor. The growth of primary tumors induces angiogenesis to assist in supplying nutrients to the tumor mass. The vasculature formed during angiogenesis facilitates the spreading of cancer cells or metastasis.¹⁵ However, the cells required an invasive capability to breach the endothelial layer and survive in the vascular system. Cancer cells can acquire this invasive property through the Epithelial-to-Mesenchymal transition (EMT) process. As the name mentioned, this process goal is acquiring the mesenchymal characteristics of the cells, in which the mobility and stress resistance of cancer cells increase. Hence, the cancer cells could survive until they reached the metastatic sites.^{1,16}

Table I Summary of TNM Cancer Staging System

Classification	Condition
Stage 0	• The presence of a group of dysplasia cells in the area
Stage 1	• Small-size cancer localized in the original organ
Stage 2	• Relatively larger in size compared to stage I • Spreading of cancer cells to nearby lymph nodes • No spreading to surrounding tissue
Stage 3	• Larger size of cancer • Spreading of cancer to surrounding tissue • Spreading of cancer cells to surrounding lymph nodes
Stage 4	• Spreading of cancer to distant organ(s) or metastatic cancer

Substantial amounts of biomarkers, including various intermediate filaments, have been studied to determine the progression and prognosis of cancer.^{8,17,18} The intermediate filament property of cancer has been used as a prognostic biomarker to assist in the determination of an appropriate therapeutic regimen for breast cancer. In addition, some types of intermediate filament have also been reported to be associated with the cancer's chemoresistance to particular chemotherapy regimens.^{19,20} Besides their role as prognostic biomarkers, the intermediate filaments have also been employed as diagnostic biomarkers and help characterize the specific type of breast cancer.^{21–23} In particular, the desmin intermediate filament has been utilized as a diagnostic marker for sarcomas, including breast sarcoma, a rare type of breast cancer. Desmin is a type III intermediate filament found in muscle and endothelial cells. Furthermore, it was used as one of the biomarkers to identify Rhabdomyosarcoma in male breast cancer patients in one of the case studies.^{24,25} Nevertheless, other intermediate filaments considerably explored in cancer studies include vimentin, nestin, and keratin, which will be discussed further in the following sections.

Vimentin

Vimentin is categorized as type III intermediate filaments. The vimentin and vimentin-related filaments are commonly found in connective tissue cells, muscle cells, and glial cells.⁶ As an intermediate filament, vimentin is crucial in cell extension formation, maintains cell integrity, and strengthens cells against mechanical stress. Vimentins also surround and protect the nucleus against deformation during a persistent invasion.^{26–28} With its various roles in diverse types of cells and tissue function, this protein is also closely related to many human diseases, including Crohn's disease, HIV, cataracts, and cancer.²⁹

In the cancer field, vimentin is often mentioned to be involved in the tumor cells' epithelial-to-mesenchymal transition (EMT) process. Vimentin is abundantly expressed in mesenchymal cells while lowly expressed in epithelial cells. Thus, it is widely known as one of the markers of EMT.³⁰ Furthermore, vimentin is closely involved in cancer metastasis, including migration, invasion, and adhesion regulation initiated by the EMT process. Vimentin promotes cell migration by inhibiting focal adhesion-associated proteins. Meanwhile, in contrast, the focal adhesion molecules will inhibit migration in the lack of vimentin expression.^{31,32} Moreover, another in vitro study on breast cancer reported that vimentin expression increased in the persistent cells upon chemotherapy. These findings indicated that the persistent cells had increased their invasiveness. Furthermore, the same study also reported that down-regulation of vimentin could reduce invasive capability while increasing sphere formation ability in MDA-MB-231 persistent cells. This outcome confirms that vimentin positively regulates the invasive capability of cancer cells.³³

Various molecular pathways involve vimentin expression. One of the often-discussed pathways related to vimentin is the PI3K/AKT pathway, which also regulates the activation of EMT transcription factors.^{34,35} Earlier studies evaluated AKT activity in cell migration and its relation with vimentin as the direct substrate of AKT. The study demonstrated phosphorylation and activation of vimentin by AKT, specifically the AKT1. The AKT1-vimentin interaction mediates the

induction of motility and invasion of tumor cells. Furthermore, the inhibition of AKT resulted in the activation of caspase 3 and vimentin proteolysis (Figure 1).³⁴

MMPs family that has been reported to increase in various cancer cells are also involved in cancer progression and metastasis.^{36,37} MMPs activity is also stated to be involved in activating EMT through the down-regulation of epithelial adhesion molecules and upregulation of the mesenchymal features in the cells. The study by Li et al discovered that the inhibition of membrane-type 1 matrix metalloproteinase (MT1-MMP) attenuates vimentin expression in cancer cells. Moreover, this condition also suppresses the invasive capability of cancer cells. Hence, it suggests the crucial role of this MMPs family in EMT through the regulation of Vimentin (Figure 1).³⁷

Furthermore, intriguing emerging exploration of the role of cell-surface vimentin (CSV) has been increasing in recent studies as a potential receptor for the development of cancer-specific targeting agents like monoclonal antibodies.^{38,39} Previous studies reported the potential role of CSV as a prognostic biomarker in detecting circulating tumor cells (CTC). The findings revealed a great potential of CSV in detecting CTC in addition to the conventional EpCAM marker for comprehensive CTC detection.^{40,41} Furthermore, a combination study on CTC detection using CSV and EpCAM as the marker to detect both epithelial and mesenchymal phenotype of CTC. The study was done to monitor breast cancer therapeutic response. The findings demonstrate the power and reliability of both methods in determining the treatment response of metastatic breast cancer patients.⁴²

As a well-known EMT marker, it is expected to evaluate the expression level of vimentin in cancer studies. Furthermore, exploring some drugs revealed some intriguing findings in how they targeted the rearrangement of vimentin in cancer. One of the active compounds that has been investigated is Withaferin A. Withaferin A is a phytocompound classified as steroidal lactones extracted from the *Withania somnifera*, known as Ashwagandha, a winter cherry plant found in India. The extract from various parts of this ancient Ayurvedic medicine has been used to treat various medical conditions, including neurological conditions such as Alzheimer's, to improve memory, enhance neurological conditions, and reduce stress.^{43–45} The extract of this plant contained various alkaloids, including Withaferin A, which has anticancer properties.^{43,46,47}

In various studies, Withaferin A (WFA) has been discovered to demonstrate cancer toxicity,⁴⁸ and inhibition of cancer proliferation,⁴⁹ angiogenesis, migration, invasion, and metastasis,⁵⁰ especially in breast cancer studies, whether as a single agent or in combination with other active compounds (Table 2). Intriguing findings demonstrated the anti-

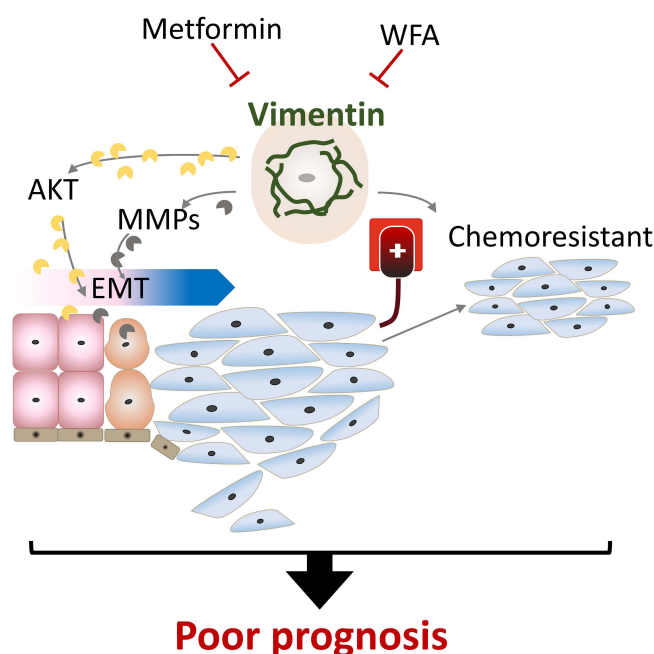


Figure 1 Vimentin involvement in cancer.

Table 2 Studies of Withaferin A and Metformin on Breast Cancer

Withaferin A (WFA)				
Type of Study	Subjects	Doses	Key Findings	Ref.
In vitro	MDA-MB-231	• Migration assay: 250 nM • Life-confocal imaging: 1 μM	• Potent anti-invasive effect • No significant effect on vimentin transcription (mRNA expression) • WFA induces Disassemble and disruption of vimentin function at the protein level • WFA increases ser56 vimentin phosphorylation	[50]
	MDA-MB-231, Mot-OE MCF7, MCF-7	• Combination WFA-CAPE (0.5 μM and 10 μM)	• WFA-CAPE combination significantly inhibits migration • WFA-CAPE increased claudin 1 expression • EMT inhibition through Wnt/β-catenin downregulation • Significant decrease of vimentin expression in both mRNA and protein levels	[51]
	MDA-MB-231	• 40 μM	• WFA represses proliferation through inhibition of K2P9 channel TASK-3	[49]
	MCF-7, SK-BR-3, MDA-MB-231	• 0.1–2 μM	• WFA inhibits osteoclast differentiation of breast cancer cells	
	MCF-10A, MDA-MB-231	• 2 μM (MCF-10A) • 2 and 4 μM (MDA-MB-231)	• WFA inhibits TNF-α/TGF-β-induced cell migration (MCF-10A) • WFA inhibits TNF-α/TGF-β-induced EMT (MCF-10A) • WFA suppresses protein expression of vimentin (MCF-10A) • WFA comparably inhibits vimentin protein expression and cell migration with vimentin knockdown group (MDA-MB-231)	[52]
In vivo	Spontaneous mouse mammary carcinoma model (4T1 cells in Balb/c mice)	• 0.1 mg/kg – 4 mg/kg (intra-peritoneal injection)	• WFA inhibits breast cancer metastasis • WFA increases ser56 vimentin phosphorylation	[50]
	Transgenic mouse models C3 (1)-SV40 Tag (FVB-Tg(C3-1-Tag) vJeg/JegJ)	• 0.16% Dietary Ashwagandha (Ash) extract (~6% WFA)	• Dietary Ash delayed the tumor development in C3 mice • Dietary Ash increased tumor suppressor P53 and P72 expression	[53]
	N-Methy-N-Nitrosourea (MNU)-induced breast cancer rat model	• 4 mg/kg or 8 mg/kg	• 8 mg/kg WFA inhibits tumor development by around 68% • 8 mg/kg WFA suppressed ALDH1 activity, one of the breast cancer stem cell biomarkers, compared to the disease control group (p=0.06)	[54]
	Female Wistar rats (benz(a)pyrene-induced breast cancer model)	• 30mg/kg b.wt weekly for 4 week	• WFA decreased serum carcinoembryonic antigen level compared to the disease control group	[55]
	MDA-MB-231 xenografts female athymic mice	• 4 mg/kg	• WFA significantly lowers vimentin protein expression in mice model	[52]

(Continued)

Table 2 (Continued).

Withaferin A (WFA)				
Type of Study	Subjects	Doses	Key Findings	Ref.
Metformin (MET)				
Type of study	Subjects	Doses	Key Findings	Ref.
In vitro	MDA-MB-231, 4T1	• 10 mmol/L	• MET inhibits EMT in both human and mouse BrCa cells through suppression of mTOR	[56]
	MDA-MB-231, MDA-MB-468, MCF-7	• 0.2, 0.4, 0.8, 1.6, 3, 2 mm	• MET suppresses mRNA expression of vimentin in MDA-MB-231 and MCF-7 cell lines • MET-resistant cells (MDA-MB-468) demonstrate greater migration capability in a dose-dependent manner • MET-resistant MDA-MB-468 expresses higher EMT markers expression (Zeb1, N-cadherin, Vimentin, and Slug protein)	[57]
	MCF-7, MDA-MB-231	• 60 mm	• MET significantly attenuates the migration of breast cancer cell lines • Co-treatment of MET and shikonin completely inhibits BrCa migration • MET suppressed the expression of pro-EMT markers and elevated an anti-EMT marker significantly in MCF-7 cells	[58]
	Tamoxifen-resistant-MCF-7	• 5 to 100 mm	• MET attenuated migration and invasion of tamoxifen-resistant BrCa cells through MMP-9 regulation	[59]
	Primary cell culture	• 5, 10, and 25 mm	• MET sensitivity on primary cancer cells showed variable IC50, ranging from 5.31 to 11.45 mm and 23.97 to 52.61 mm • 10 mm MET decreases Vimentin and SNAIL protein expression in a time-dependent manner • MET reverted primary cancer cells IL-6-induced EMT	[60]
	MDA-MB-231	• 100 µM	• MET inhibits cell migration by 63% • MET inhibits cell invasion by 40%	[61]

In vivo	Allograft (4T1) mammary tumor BALB/c mice	• 200 mg/kg/day (intraperitoneal)	• MET significantly inhibits the development of primary tumors in allograft mice model • MET single treatment does not affect BrCa metastatic to the lung, yet MET significantly suppressed metastasis in DPP-4 inhibitor KR62436-induced EMT	[56]
	Xenograft (MDA-MB-231) BALB/c-nu nude mice model	• 22mM	• MET significantly suppressed vascular formation on Xenograft nude mice model • MET upregulates STAT3 protein expression, while significantly suppressed MMP-2, MMP-9, IL-6, and IL-7 expression	[62]
	Allograft (4T1) BALB/c and Xenograft (MDA-MB-231) BALB/c-nu mice	• Low dose: 0.1, 1, and 25 mg/kg/day • Clinically relevant (CR) dose: 225 mg/kg/day	• CR dose MET greatly decreases allograft cancer growth by 63.7% • CR dose MET significantly reduces the rate of lung metastasis by 53.9% • MET pretreatment elevates chemotherapy drug sensitivity • MET attenuate angiogenesis	[63]

Abbreviations: CAPE, caffeic acid phenethyl ester; CR, clinically relevant; MET, metformin; WFA, Withaferin A.

migration effect of WFA on breast cancer cells. The study showed that WFA suppressed cell migration while not suppressing vimentin expression, as indicated by the insignificant difference in vimentin mRNA expression in the cells treated by WFA. However, microscopic observation showed less distinct formation of lamellipodia and vimentin formation in the leading edge of the cells of WFA-treated cells. This finding suggests that vimentin's function in forming lamellipodia has been disrupted through the disassembly of vimentin by the interaction of WFA and vimentin proteins.⁵⁰

Another active compound with intriguing effects on vimentin in cancer cells is metformin. This first-line drug commonly used for Type-2 diabetes patients, metformin, has emerged in cancer studies with its positive impact on cancer. Metformin itself is regarded as the first-line treatment for most type-2 diabetes patients.⁶⁴ Various findings reported the favorable effect of metformin on cancer therapy, which includes the antiproliferative effect,^{65,66} anti-migration,⁶⁷ inhibition of EMT,⁶⁸ and its potential to assist cancer immunotherapy.⁶⁹

A study on metformin reveals that it inhibits the migration of endometrial cancer cells through the PI3K/AKT/MDM2 pathway.⁶⁷ Furthermore, this drug attenuates the EMT process of endometrial cancer by affecting the intermediate filament expression, including the upregulation of Pan-keratin and suppression of vimentin.⁶⁸ Other studies by Valee demonstrate that metformin suppresses the EMT process in gastric cancer.⁷⁰ Metformin is also reported to exhibit an inhibition effect on cancer cell stemness and EMT by inhibiting the Wnt3a/ β -catenin pathway, by which one of the downstream is the expression of vimentin.^{71,72} Further studies discovered that metformin specifically targeting vimentin has a comparable suppression effect with vimentin-siRNA.^{70,73}

Nestin

Nestin cytoskeleton is classified as Type IV intermediate filament, initially typically identified in the neuroepithelial progenitor/stem cell in the earlier year.⁷⁴ Hence, nestin has been known as a biomarker for central nervous system progenitor cells. Despite this, further studies also demonstrate the expression of nestin in other types of progenitor cells, including endothelium, testes, teeth, bone marrow, intestines, muscles, and other tissues.^{75–81} In-depth studies by Sahlgren et al describe the involvement of nestin in cell regulation, specifically related to its phosphorylation by CDK1 (cdc2 kinase) and CDK5. The rearrangement of nestin was reported to stimulate cell proliferation and survival against oxidative stress-induced cell death (Figure 2).^{82–84}

Li et al published the first identification of nestin expression in breast cancer. It was found specifically in the basal layer of highly regenerative cells of normal mammary glands. Nestin expression was colocalized with other

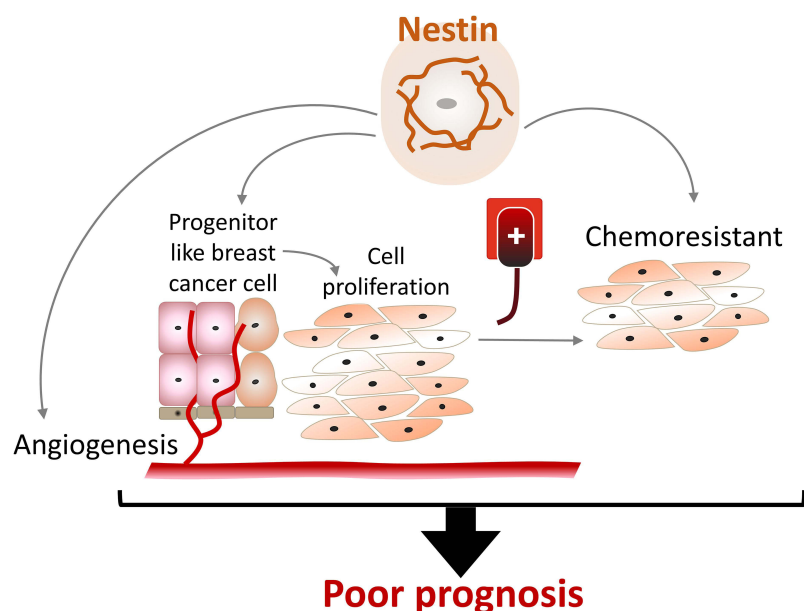


Figure 2 The role of nestin in breast cancer progression.

myoepithelial or basal epithelial markers, such as p63 and CK14—similar protein profiles in basal-like breast cancer cells, including high nestin, p63, and CK14 expression. In another study, nestin expression is also abundantly found in BRCA1-related breast cancer. Hence, it demonstrates the phenotype of a progenitor-like cell of a BRCA1-related tumor with high aggressiveness and reduced overall survival of the specific breast cancer.^{85,86}

Nestin was also reported to be involved in drug resistance to breast cancer. A study by Feng et al demonstrates the correlation of a long noncoding RNA (lncRNA) ENST869 with nestin expression in breast cancer cells. In the study, the inhibition of lncRNA ENST869 by siRNA3 resulted in the downregulation of nestin expression. Moreover, the decrease of nestin led to the elevation of an anticancer drug, Chidamide, in its effectiveness on breast cancer cells, implying nestin's role as a crucial drug resistance factor in breast cancer.²⁰

Another study on breast cancer reported that nestin is specifically expressed during the development of the endothelium of blood vessels. Thus, high nestin expression is found in microvessels during angiogenesis. Furthermore, high nestin expression microvessel density (Nestin⁺MVD) was related to shorter overall survival (OS) in breast cancer patients. Hence, Nestin⁺MVD is proposed to be an independent prognostic factor in breast cancer.¹⁷ A follow-up study further confirmed the importance of nestin as a poor prognostic factor for breast cancer, in which the expression of nestin in both mRNA and protein levels is elevated in more aggressive types of breast cancer cell lines.¹⁸

Keratin

Keratin or cytokeratin (CK) is classified into Type I and Type II intermediate filaments of epithelial cells, which assemble into filament structures by heterodimerization and polymerization.^{6,87} Fifty-four human keratins have been characterized, which consist of 37 human epithelial keratins and 17 hair keratins. Type I epithelial keratins consist of 17 subclasses (CK9-CK28), in which numbers CK11, CK21, and CK22 are not used currently due to some circumstances discovered before. Meanwhile, Type II epithelial keratins are classified into 20 subclasses (CK1-CK8 and CK71-CK80), in which CK6 consists of three variables: CK6a, CK6b, and CK6c.^{88–90}

As a part of the cytoskeleton family, keratins are an integral factor in maintaining the structural stability of epithelial cells. Furthermore, the importance of keratin filament is beyond the cellular level due to its interaction with desmosomes and hemidesmosomes, which provide stability for intercell connection and the attachment of the cells to the basal membrane.⁹¹ In cancer studies and clinical settings, keratin pattern determination has been extensively utilized to characterize tumors, including metastasis tumors. This exploitation occurs due to the general preservation of keratin pattern expression in tumor cells mimicking the origin cells it derived from.^{91,92} Cytokeratin subclasses, which are substantially expressed and used in breast cancer typing, include CK5/CK6, CK7, CK8, CK17, CK18, and CK19 (Figure 3).^{7,91,93}

Cytokeratin 5 (CK5) and cytokeratin 6 (CK6) are often identified concurrently using bispecific antibody CK5/CK6 for clinical cancer diagnostic purposes, even though they are not functionally associated.^{21,88} Both cytokeratins are typically expressed in squamous cell types. The CK5 is mainly localized in the basal layer, while the CK6 is mostly expressed in the suprabasal layers. Hence, the identification of CK5/CK6 is utilized to determine the origin of the carcinoma's squamous cell type in weakly differentiated carcinomas.^{21,22} In breast cancer studies, CK5/CK6 is often used as a biomarker to identify the subtype of triple-negative breast cancer (TNBC) with basal-like type.^{94,95} One of the studies showed that patients with CK5/CK6-positive breast cancer have relatively shorter overall survival compared to patients with negative CK5/CK6. Furthermore, maintenance treatment using capecitabine does not increase the overall survival of the CK5/CK6 positive compared to the CK5/CK6-negative group. Thus, CK5/CK6 can serve as a prognostic biomarker, which identifies the poorly prognosis breast cancer.^{21,22}

Cytokeratin 7 (CK7) expression in normal conditions is related to various normal cellular physiological functions such as proliferation, migration, and EMT during embryogenesis. In the same regard, the high level of CK7 expression is also associated with proliferation, migration, and EMT processes in cancer progression.⁸ As a prognostic biomarker, the higher expression of CK7 is associated with poorer outcomes for the patient. Nevertheless, studies on the attenuation of CK7 expression, such as using the long non-coding RNA CK7 antisense, revealed a tumor-suppressive effect on breast cancer.^{8,96} The presence of CK7, along with the absence of CK20, has been employed in identifying various original cancer sites of metastase carcinoma, including breast cancer and several other original sites.⁹⁷ Due to the low specificity

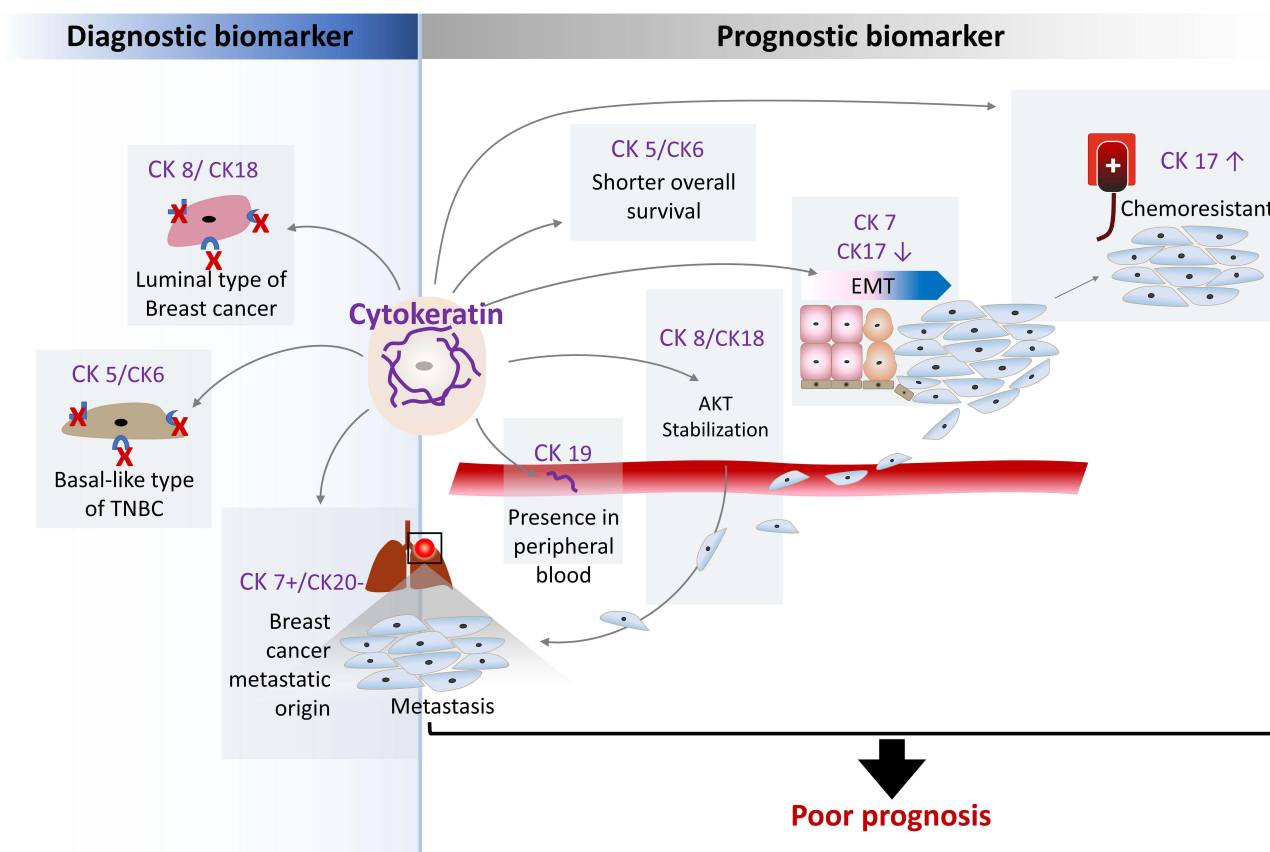


Figure 3 The role of cytokeratin in breast cancer progression.

of the CK7+/CK20-profile to identify breast cancer origin of metastases tumors, combination evaluation with other biomarkers as comprehensive diagnostic tools for breast cancer metastases cancer has been studied.²³

Cytokeratin 8 (CK8) forms an obligate heterodimer with cytokeratin 18 (CK18). CK8/CK18 is dominantly expressed in the luminal type of breast epithelial cells. Thus, it is often used to identify the luminal type of breast cancer.⁹⁸ The overexpression of CK8/CK18 heterodimer was reported to induce various unfavorable effects on cancer progression and treatment. In earlier years, it was discovered that overexpression of CK8/CK18 leads to multiple drug chemoresistant in cancer.^{19,99,100} These cytokeratin dimers also demonstrate the protection effect of breast cancer against apoptosis through the TNF-related apoptosis-inducing ligand pathway.¹⁰¹ CK8/CK18 also acts as a substrate of Akt, stabilizing Akt and possibly assisting cancer progression and metastases. Meanwhile, the phosphorylation of CK18 of the CK8/CK18 heterodimer inhibits Akt-CK8/18 protein interaction.¹⁰²

Cytokeratin 17 (CK17) can be found in the basal type of epithelial cells, which regulate many physiological processes of the cells, including cell growth and proliferation.¹⁰³ Breast cancer with CK17-positive is usually associated with increased cancer aggressiveness and chemoresistant or low sensitivity against chemotherapy drugs, especially in the TNBC subtype. Suppression of CK17 leads to increased sensitivity of doxorubicin-resistant breast cancer towards doxorubicin treatment. However, the loss of CK17 also promotes the activation of EMT and the acquirement of mesenchymal cells characteristic of breast cancer.^{104,105} Interestingly, the opposite role of CK17 is found in HER2^{HIGH} breast cancer, in which the suppression of CK17 expression leads to poor prognosis of the disease.¹⁰⁶

Cytokeratin 19 (CK19) is the tiniest size of cytokeratin protein with myriad pattern expressions found in various cells, including muscle cells, epithelial cells, and epithelial stem cells.^{88,107} The expression of CK19 is essential for cell proliferation and has also been reported to be correlated with poor prognosis in several types of cancer.^{108,109} Intriguingly, the role of CK19 as a prognosis biomarker in breast cancer is still debatable. The expression of CK19 is

needed in luminal breast cancer cells to proliferate and regulate the cell cycle by interacting and stabilizing cyclin D3.¹¹⁰ The expression of CK19 is often evaluated in its soluble state and is utilized to monitor the development of breast cancer.^{91,93,111} The peripheral blood of a normal person does not contain CK19 mRNA.

Meanwhile, the presence of CK19 mRNA is found in almost 50% of non-metastatic breast cancer and over 50% positive rate for the subjects with metastatic breast cancer. Thus, it is correlated with the poor outcome.¹¹² However, another study reveals a fascinating finding: the expression of CK19 in aggressive breast cancer is lower than in the less aggressive type of breast cancer. Furthermore, the study demonstrates that cancer cells are inhibited in cell proliferation, migration, and chemoresistance.¹¹³

Last but not least, there are emerging discussions on the role of cell-surface keratin (CSK) in cancer progression. Previous studies reported that the expression of cell-surface keratin 8 (CK8) is involved in the mechanism by which cancer cells evade immune surveillance. The presence of CK8/CK18 or CK8/CK19 on the surface of metastatic carcinoma cells concealed the major histocompatibility complex class I (MHC I) molecules, thus avoiding elimination by cytotoxic T-cells due to MHC I – T-cell receptor (TRC) interaction inhibition.^{3,114,115}

Another intriguing cell surface keratin that has been studied is cell-surface keratin 1 (CK1). This molecule was found to be overexpressed in malignant cells. Previous research on breast cancer developed cytotoxic peptides that specifically targeted CK1 in breast cancer cells, known as peptide 18–4, the analog of cytotoxic peptide p160. Both p160 and its analog peptide 18–4 could internalize into the cancer cells through the binding with CK1, while peptide 18–4 does not affect the viability of cancer cells.¹¹⁶ Recent studies by Ziaei et al revealed the development of peptide-drug conjugated, which targets keratin 1 in triple-negative breast cancer cells and the tumor xenograft mice model. The team developed conjugation of peptide 18–4 with doxorubicin (Dox). The peptide 18–4–Dox conjugated demonstrated high cytotoxicity comparable with free Dox on TNBC cells.¹¹⁷ Furthermore, the TNBC xenograft model displayed a greater accumulation of Dox in the peptide-Dox conjugated group in the breast cancer tissue compared to the group treated with free Dox. Meanwhile, the accumulation of Dox in other normal tissues, including the liver, heart, and lungs, is significantly lower for the peptide-Dox group compared to the free Dox group. Thus, these findings imply the potential of CK1 as a receptor for targeted therapy of TNBC.^{118,119}

Conclusion

In cancer studies, the role of intermediate filament, specifically vimentin, nestin, and keratin, is often mentioned as various biomarkers, whether diagnostic or prognostic. Various intermediate filaments are frequently cited to be related to the progression of cancer. Besides its role in determining the appropriate treatment procedure for the patients, the role of these intermediate filaments as target therapy is also an intriguing topic to explore. The overexpression of vimentin and nestin generally indicates a poor prognosis of cancer. However, due to the myriad subclass of keratin and evidence showing high or low expression of keratin in breast cancer, no conclusion can be drawn for keratin expression in general. In some cases, some keratin subclasses were found to be overexpressed in breast cancer, including CK5/CK6, CK7, CK8/CK18, and CK19, which are involved in angiogenesis promotion, metastasis, and chemoresistance.^{98,102,105,112,120} On the other hand, some studies also reported a protective effect of keratin 17 against cancer.¹⁰⁶ Furthermore, other studies on keratin, such as CK19 were found to be downregulated in highly aggressive breast cancer compared to less aggressive cancers.¹¹³

The range of promising anticancer drug research has increased, encompassing natural-derived bioactive compounds and chemical synthesis products. The exploration of potential anticancer drugs even reaches the exploration of other medications used to treat other ailments. Most potential substances were evaluated for their cytotoxicity, antiproliferative, and anti-metastasis effects on cancer cells. Some studies on active compounds, such as Withaferin A and Metformin, demonstrated the substance's impact on vimentin in cancer cells concerning the cancer progression and anti-metastasis effect.^{43,46,49,65,69,73,121} In addition, exploration in peptide-drug conjugation targeting cell-surface keratin 1 displayed the potential of cell-surface keratin as a receptor, especially for TNBC therapy development.^{116,117,119} However, the effect of active compounds on other intermediate filaments, such as nestin, is still underexplored.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, data analysis and interpretation, or all these areas; took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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