FUNCTIONAL ROLE OF VIMENTIN'S CYSTEINE IN XIST-MEDIATED EMT INHIBITION IN BREAST CANCER

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ABSTRACT

Breast cancer is the most commonly diagnosed malignancy among women worldwide and a leading cause of cancer-related mortality, primarily due to its high metastatic potential. One mechanism underlying metastasis is the epithelial-to-mesenchymal transition (EMT), which enhances cancer cell mobility, invasiveness, and resistance to treatment. Vimentin, a type III intermediate filament protein, is a hallmark of EMT and plays a structural and regulatory role in cytoskeletal organization and cellular stress responses. Recent studies have highlighted the importance of a single cysteine residue at position 328 (C328) in vimentin, which functions as a redox-sensitive site influencing filament dynamics. However, the role of C328 in cancer progression remained largely unexplored. This literature review investigates the effect of a single amino acid substitution—C328 to serine (C328S)—on breast cancer cell behavior, focusing on findings published between 2020 and 2025, sourced from PubMed and Google Scholar. Evidence from MCF-7 breast cancer cell models reveals that expression of C328S-VIM induces morphological changes, cytoskeletal disorganization, and increased proliferation, migration, and invasion. Notably, C328S-VIM upregulates the long non-coding RNA XIST, which promotes EMT, estrogen independence, and stem-like properties. These findings indicate that the native C328 residue serves a tumor-suppressive function, partly through modulation of XIST activity. Overall, this review presents a novel insight into how a single amino acid mutation in vimentin can reprogram breast cancer cells toward a more aggressive and stem-like phenotype. The study highlights C328 as a potential therapeutic target and broadens our understanding of the molecular mechanisms driving breast cancer progression.

Keywords : Breast cancer, vimentin, XIST, intermediate filament protein, EMT

INTRODUCTION

Breast cancer is the most prevalent malignancy among women worldwide and represents a leading cause of mortality, primarily due to its high metastatic potential (Wilkinson & Gathani, 2022). Metastasis may still occur even after the administration of adequate treatment. One of the underlying mechanisms is the epithelial-to-mesenchymal transition (EMT), during which cells acquire a more aggressive phenotype and enhanced migratory capacity (Talbot et al., 2012; Yeung & Yang, 2017). Vimentin is a type III Intermediate Filament (IF) protein found in mesenchymal cells and plays a role in various

cellular and pathophysiological processes, including Epithelial-Mesenchymal Transition (EMT) and cancer metastasis (Liu et al., 2015; Kuburich et al., 2023).

Vimentin is predominantly found in cells of mesenchymal origin, where it forms an extensive, dynamic, and highly elastic filamentous network. Additionally, vimentin has a dynamic structure that supports various cellular functions, ranging from nuclear stability to immune responses (dos Santos et al., 2015; Patteson et al., 2019; Arrindell & Desnues, 2023). Vimentin plays a role in various processes involving the cytoskeleton, such as maintaining cell shape, forming focal adhesions, extending lamellipodia, building stress fibers, and transmitting signals. Although its crystal structure has not been fully elucidated, its polymerization process is known to occur gradually, forming mature filaments that contribute to cellular mechanical integrity and interactions with actin (Pradeau-Phélut & Etienne-Manneville, 2024).

The cysteine residue C328 in the rod domain of vimentin functions as a stress sensor in response to oxidants and electrophiles, triggering zinc-based modifications and leading to filament changes (Pérez-Sala et al., 2015; Mónico et al., 2021; González-Jiménez et al., 2023). C328 modification occurs under various conditions, such as cellular aging, rheumatoid arthritis, cataracts, and atherosclerosis (González-Jiménez et al., 2023; Pajares & Pérez-Sala, 2024). Changes in C328 can disrupt cellular stress responses, impact cell function, and play a role in various pathophysiological processes. However, its role in EMT, tumor growth, and cancer progression remains largely unexplored (González-Jiménez et al., 2023).

To understand the role of the C328 residue of vimentin in EMT, cancer progression, and stemness properties, vimentin C328S was expressed in MCF-7 cells. The study results showed that C328S-VIM induced changes in cell morphology, disrupted F-actin, and promoted EMT and cancer stem cell characteristics. Additionally, C328S-VIM expression increased IncRNA XIST, making MCF-7 cells, which are normally estrogen-dependent, become estrogen-independent in a mouse model. Overall, C328S-VIM plays a role in EMT and stemness, possibly through the upregulation of XIST, which is relevant to various vimentin variants in solid tumors (Usman et al., 2025).

This review discusses recent advancements in the field of stem cell research, specifically highlighting the discovery that stem cells may arise from a disruption involving a single amino acid in a cellular protein found in breast cancer cells. The purpose of this review is to elaborate on the core issue and present it as a potential new insight in the field of regenerative medicine.

MATERIALS AND METHODS

This study is a literature review aimed at examining the impact of a single amino acid alteration in a cellular protein on the transformation of cancer cell behavior into a cancer stem cell-like phenotype. The review was conducted by analyzing relevant scientific literature published between 2020 and 2025. All selected articles were written in English. The literature sources were obtained from two primary databases, PubMed and Google Scholar, using a combination of keywords such as: "amino acid mutation," "cancer stem cell," "protein alteration in cancer," "single amino acid change," and "cancer cell behavior." Boolean operators such as AND and OR were applied to combine keywords and broaden the scope of relevant search results. This stage of writing is carried out by the literature search method, which is sourced from online searches, through Google Scholar, Pubmed, and officially published articles. The data processing stage starts from screening

keywords that match the chosen topic, then proceeds to draw conclusions based on the data obtained and then narrowed down to get a conclusion.

As an alternative to the PRISMA framework, this study employs a simplified four-stage screening approach, structured as follows:

1. Identification
   All articles retrieved using the specified keyword combinations were recorded, including metadata such as title, author(s), year of publication, and journal name.
2. Preliminary Screening
   The titles and abstracts of each article were reviewed to evaluate their relevance to the focus of the study. Articles that did not address amino acid mutations or changes in cancer cell behavior were immediately excluded.
3. Full-Text Review
   Articles that passed the preliminary screening were then examined in full. At this stage, only studies that explicitly discussed the relationship between a single amino acid mutation in a cellular protein and changes in cancer cell properties or behavior resembling that of stem cells were selected for further analysis.
4. Final Selection
   Articles were selected based on content relevance, scientific quality (peer-reviewed publications), and their contribution to understanding relevant molecular mechanisms. Duplicate entries and studies with invalid or unreliable data were excluded.

The selected literature was analyzed qualitatively to explore patterns in molecular mechanisms, signaling pathways, and the biological implications of single amino acid mutations in the context of cancer cell behavioral transformation. These findings were then synthesized to develop a comprehensive understanding of the topic under investigation.

RESULT AND DISCUSSION

Recent studies have shown that the C328S mutation in vimentin plays a role in suppressing EMT, based on comparisons between C328S-VIM and WT-VIM cells. Cells expressing C328S-VIM exhibit morphological changes, increased proliferation, enhanced mitochondrial activity, and greater migratory and invasive capabilities (Usman et al., 2025). These findings suggest that the mutation promotes a more aggressive cancer cell phenotype.

Vimentin is an important protein in cells, which contains a region known as the cysteine residue at position 328 (C328). This region often undergoes changes after the protein is formed, known as post-translational modification (Kaus-Drobek et al., 2020). To date, no research has specifically investigated the role of C328 in cancer and whether it affects the characteristics of cancer stem cells.

A recent experiment by a research team involved replacing the C328 residue (cysteine) with serine, resulting in a new protein called C328S-VIM. This protein was then introduced into MCF-7 breast cancer cells, which naturally lack vimentin (Sivagurunathan et al., 2022). The results showed that C328 is a crucial component of the vimentin protein. Its substitution caused disruptions in cell structure, altered cell morphology, and potentially affected the cancer cells' ability to spread (González-Jiménez et al., 2023). This experiment suggests that small changes at specific points in the protein can significantly influence cancer cell behavior (Usman et al., 2025).

The C328S mutation in vimentin causes cancer cells to grow faster, move more easily, and penetrate other tissues more effectively, while reducing their ability to stick to one another—all of which are hallmarks of highly metastatic cancer. This suggests that the original C328 site in vimentin may actually function to suppress cancer progression (Usman et al., 2025). The mutation also increases the expression of markers typically found in breast cancer stem cells, indicating that the cells become more stem-like and potentially more dangerous. Furthermore, the C328S mutation shifts MCF-7 cancer cells from being "triple positive" (a subtype with better treatment outcomes) to "triple reduced," which is more difficult to treat (Winter et al., 2021). Previously, the normal version of vimentin did not affect this trait, suggesting that the change is likely due to a disruption in the interaction between vimentin and other cellular proteins, ultimately promoting cancer growth and spread (Strouhalova et al., 2020).

One of the genes most upregulated as a result of the C328S mutation is XIST, a type of non-coding RNA that does not produce proteins but still plays a crucial role (Usman et al., 2025). XIST has previously been shown to accelerate breast cancer cell growth, promote metastasis, and prevent normal cell death. This gene also helps maintain the balance of normal breast tissue, particularly by regulating stem cell development (Zong et al., 2020; Ma et al., 2023). When vimentin is mutated to C328S, XIST becomes more active and triggers changes resembling the EMT process, making cancer cells more aggressive. However, when XIST activity is reduced, the growth and invasiveness of C328S-mutant cells also decrease (Zong et al., 2020). This indicates that the mutation promotes more malignant cancer behavior through XIST upregulation, but its effects can be mitigated if XIST is blocked (Usman et al., 2025).

Traditionally, vimentin has been characterized as a biomarker of epithelial–mesenchymal transition (EMT) and metastatic potential in cancer. However, emerging evidence indicates that vimentin also exerts a functional role in tumor progression (Usman et al., 2021). Specifically, the cysteine residue at position 328 (C328) has been shown to regulate actin cytoskeletal dynamics and suppress EMT and tumorigenic behaviors through modulation of the long non-coding RNA XIST (Yang et al., 2021). These findings highlight a previously unrecognized mechanistic link between vimentin and transcriptional regulation in cancer cells and suggest that targeting vimentin, particularly at C328, may offer a novel therapeutic avenue for breast cancer treatment.

SUMMARY

Recent research has revealed that a mutation in the vimentin protein, specifically the substitution of cysteine at position 328 with serine (C328S), enhances aggressive cancer traits in breast cancer cells. This mutation leads to increased cell proliferation, invasion, and stem-like properties, while reducing cell adhesion—key features of metastatic cancer. The C328 residue normally helps suppress these behaviors, partly by regulating the activity of XIST, a non-coding RNA linked to tumor growth and EMT. When mutated, XIST becomes overactive, promoting malignancy. These findings suggest that the C328 site in vimentin plays a critical role in controlling cancer progression and may serve as a potential target for breast cancer therapy.

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