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Review

Exosomal misfolded proteins released by cancer stem cells: dual functions in balancing protein homeostasis and orchestrating tumor progression

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Abstract
Cancer stem cells (CSCs), the master regulators of tumor heterogeneity and progression, exert profound influence on cancer metastasis, via various secretory vesicles. Emerging from CSCs, the exosomes serve as pivotal mediators of intercellular communication within the tumor microenvironment, modulating invasion, angiogenesis, and immune responses. Moreover, CSC-derived exosomes play a central role in sculpting a dynamic landscape, contributing to the malignant phenotype. Amidst several exosomal cargoes, misfolded proteins have recently gained attention for their dual functions in maintaining protein homeostasis and promoting tumor progression. Disrupting these communication pathways could potentially prevent the maintenance and expansion of CSCs, overcome treatment resistance, and inhibit the supportive environment created by the tumor microenvironment, thereby improving the effectiveness of cancer therapies and reducing the risk of tumor recurrence and metastasis. Additionally, exosomes have also shown potential therapeutic applications, such as in drug delivery or as biomarkers for cancer diagnosis and prognosis. Therefore, comprehending the biology of exosomes derived from CSCs is a multifaceted area of research with implications in both basic sciences and clinical applications. This review explores the intricate interplay between exosomal misfolded proteins released by CSCs, the potent contributor in tumor heterogeneity, and their impact on cellular processes, shedding light on their role in cancer progression.

Keywords Exosomes · Cancer stem cells · Misfolded proteins · Drug resistance · Proteostasis · Metastasis

Abbreviations

ABC	ATP-Binding cassette
AbTAC	Antibody-based PROTAC
ATF4	Activating transcription factor 4
ATF6	Activating transcription factor 6
Bcl2	B-cell lymphoma 2
CSCs	Cancer stem cells
DDR	DNA damage response
eIF2α	Eukaryotic initiation factor 2α
ER	Endoplasmic reticulum
ERAD	Endoplasmic reticulum-associated degradation

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GADD34	Growth arrest and DNA damage-inducible protein 34
GRP78	Glucose-regulated protein 78
HSPs	Heat-shock proteins
IRE1	Inositol-requiring enzyme 1
JNK	Jun N-terminal kinase
LYTAC	Lysosome-Targeting Chimaera
PDIs	Protein disulphide isomerases
PERK	Protein kinase R-like ER kinase
PROTAC	Proteolysis Targeting Chimeras
SOX2	SRY (sex determining region Y)-Box 2
TPD	Targeted protein degradation
TRAF2	TNF receptor-associated factor 2
UPR	Unfolded protein response
XBP1	X-box binding protein 1

1 Introduction

Cancer stem cells (CSCs) are a unique subpopulation of cells within tumors which have garnered significant attention due to their exceptional capacity for self-renewal and differentiation. These abilities are pivotal in driving tumor heterogeneity and conferring resilience against conventional therapies, rendering CSCs a critical focus for cancer research and therapeutic development [1]. The complex interplay between CSCs and their microenvironment is further compounded by recent discoveries highlighting the role of exosomes in mediating intercellular communication. Exosomes are specialized extracellular vesicles, typically 30 to 150 nm in diameter, that transport a diverse array of molecular cargo, including proteins, lipids, various RNAs, and DNA fragments, and reflect the signature components of the originating cell [2].

This review centers on an underexplored but significant aspect of the exosomal cargo, the misfolded proteins. These proteins, often produced under conditions of cellular stress and proteostatic challenges, are generally managed by the protein quality control machinery of the cell. However, when this machinery is overwhelmed, misfolded proteins accumulate, potentially disrupting cellular functions and contributing to disease states [3]. While the role of exosomal misfolded proteins in neurodegenerative diseases is well-documented [4], their impact in cancer biology, particularly in the context of CSCs, is yet to be elucidated.

CSCs are silent yet essential players for cancer progression due to their involvement in tumor initiation, progression and recurrence. Understanding the mechanisms related to protein homeostasis is essential for developing more effective therapeutic strategies targeting these resilient cells [5]. The misfolded proteins residing in CSCs play a multifaceted role in cellular processes. Within the CSC population, these proteins can disrupt the proteostasis network, leading to the loss of stemness and stimulating differentiation into progenitor cells [6]. However, CSCs exhibit an extraordinary ability to adapt to proteostatic stress, ensuring their survival and functionality through a robust protein quality control system. This adaptability highlights the dynamic and resilient nature of CSCs in overcoming challenges associated with protein folding and function [7].

The exosomal transfer of misfolded proteins adds another layer of complexity to the biology of the CSCs. Research suggests that while these proteins help CSCs adapt to proteostatic stress, they may also promote carcinogenesis in recipient cells [8]. This dual role underscores the potential of exosomal misfolded proteins to act as both facilitators of proteostatic adaptation and instigators of tumor development and metastasis [9]. The ability of misfolded proteins to propagate proteotoxic stress and malignancy, when delivered through exosomes, raises concerns about the unintended consequences of this form of intercellular communication [10].

Targeting the proteostasis mechanisms and exosomal pathways involved in the transfer of misfolded proteins could disrupt CSC survival, reduce tumor heterogeneity, and hinder metastasis [11]. By interfering with the processes that enable CSCs to thrive despite proteostatic stress, it may be possible to enhance the efficacy of existing treatments and overcome drug resistance. This approach not only addresses the survival mechanisms of CSCs but also targets the intercellular communication pathways that facilitate tumor progression and metastasis [12]. The potential to develop biomarkers based on the presence of specific misfolded proteins in exosomes further underscores the clinical relevance of this research, offering new avenues for early cancer detection, monitoring disease progression, and predicting treatment response [13], ultimately reducing tumor burden and improving clinical outcomes [14].

2 Role of cancer stem cells in tumor progression

CSCs, which represent a small subpopulation of cells within tumors that exhibit stem cell-like properties [15], was first identified in hematological malignancies [16], and have since been found in a variety of solid tumors, including breast [17], brain [18], colon [19] and pancreatic cancers [20]. These cells are crucial drivers of tumorigenesis due to their unique ability to self-renew, differentiate into multiple cell types, and exhibit resistance to standard cancer therapies.

2.1 Self-renewal and differentiation

CSCs have the capacity for self-renewal, enabling them to maintain the CSC pool and sustain long-term tumor growth [21]. They can also differentiate into diverse cell types that constitute the tumor bulk, contributing to the cellular heterogeneity observed within tumors. This heterogeneity is a significant factor in the adaptive capability of tumors to survive and proliferate under different microenvironmental conditions. The plasticity of CSCs allows them to dynamically switch between different cellular states, promoting tumor growth and evolution [22].

2.2 Tumor initiation and growth

CSCs are often considered the root of tumor initiation due to their ability to give rise to new tumors when transplanted into immunocompromised mice. Their high tumorigenic potential is attributed to their stem-like properties, which enable them to efficiently propagate the tumor. In many cancers, the presence of CSCs correlates with a higher grade of malignancy and poor clinical prognosis. The ability of CSCs to adapt and survive in various microenvironments makes them critical players in tumor persistence and expansion [15].

2.3 Metastasis and invasion

CSCs are implicated in metastasis. They exhibit enhanced motility and invasiveness, allowing them to disseminate from the primary tumor and establish secondary tumors at distant sites. CSCs often express markers and signaling pathways associated with epithelial-to-mesenchymal transition (EMT), a process that endows them with increased migratory and invasive capabilities. This transition is crucial for the dissemination of CSCs, enabling them to invade surrounding tissues and enter the bloodstream, facilitating metastasis [23].

2.4 Therapy resistance

CSCs are inherently more resistant to conventional chemotherapy and radiation compared to the bulk of the tumor cells. This resistance is mediated by several mechanisms, including activation of drug efflux pumps, enhanced DNA repair capabilities, and a quiescent cell cycle state, that makes them less susceptible to treatments targeting rapidly dividing cells [24]. Additionally, CSCs can undergo symmetric and asymmetric cell divisions, contributing to both maintenance of the CSC population and the production of therapy-resistant progeny.

2.5 Tumor relapse

CSCs are the main players behind cancer relapse. After conventional therapies reduce the bulk of the tumor, CSCs can survive due to their resistance mechanisms and repopulate the tumor, leading to recurrence. The persistence of CSCs post-therapy can lead to tumor relapse and metastasis. The dormant state of some CSCs allows them to evade treatment and later reactivate, initiating new tumor growth. This ability to remain dormant and later reactivate makes targeting CSCs particularly challenging and underscores the need for therapies specifically aimed at eradicating CSCs to prevent relapse [25].

2.6 Signaling pathways and microenvironment

Various signaling pathways, including Wnt, Notch, Hedgehog, and PI3K/Akt, are crucial in maintaining CSC properties and promoting their survival [26]. The tumor microenvironment, which includes cancer-associated fibroblasts, immune cells, and extracellular matrix components, also plays a significant role in supporting CSC function and plasticity [27]. This microenvironmental support helps CSCs evade immune surveillance and adapt to changing conditions within the tumor niche. The interaction between CSCs and their niche is a dynamic process that influences tumor progression and response to therapy.

2.7 CSC-derived molecules within the tumor microenvironment

CSC-derived secretions, including damage-associated molecular patterns (DAMPs), cytokines, and other signaling molecules, play a pivotal role in shaping the tumor microenvironment (TME) during tumor progression. DAMPs, such as high mobility group box 1 (HMGB1) and heat shock proteins (HSPs), are released by CSCs in response to cellular stress and necrosis, acting as potent immunomodulators that can attract and activate immune cells [28]. However, CSCs often exploit this response to create an immunosuppressive milieu by recruiting regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs), which inhibit effective anti-tumor immunity. CSCs also secrete various cytokines, such as interleukins (e.g., IL-6, IL-8) and transforming growth factor-beta (TGF-β), which promote inflammation, angiogenesis and epithelial-to-mesenchymal transition (EMT), facilitating tumor invasion and metastasis. Additionally, these cytokines can induce differentiation of fibroblasts into cancer-associated fibroblasts (CAFs), which remodel the extracellular matrix (ECM) and support tumor growth [29]. CSCs not only create a supportive niche for their own maintenance and self-renewal but also actively remodel the TME to favor tumor progression, immune evasion and metastatic spread by secreting specific molecules.

Comprehending the molecular mechanisms governing CSC functions is therefore essential for developing targeted therapies aimed at eradicating these cells and achieving long-term remission in cancer patients. Advances in CSC research hold the promise of novel therapeutic strategies that specifically target CSCs, thereby improving patient outcomes and reducing the incidence of tumor relapse.

3 Maintenance of protein homeostasis in cancer stem cells

3.1 Proteostasis

Cells have evolved a sophisticated set of mechanisms to ensure the maintenance of protein homeostasis (proteostasis). Proper folding of proteins is essential for their specific biological activity within a cell [30]. Proteins are synthesized as linear chains of amino acids. The folding process is intricate and precise, governed by the amino acid sequence and influenced by various cellular factors [31]. The cellular proteostasis network includes chaperone proteins, folding enzymes, and quality control systems, working together to facilitate the correct folding of proteins and to eliminate misfolded or damaged proteins [32].

3.1.1 Role of chaperone proteins

Chaperone proteins play a crucial role in cellular processes by facilitating the correct folding of newly synthesized polypeptide chains and aiding in the refolding of misfolded proteins [33]. The proper three-dimensional structure of a protein is essential for its functional activity, and chaperones ensure that this structure is achieved accurately. One of the primary roles of chaperones is to prevent the aggregation of unfolded or partially folded proteins, which could lead to the formation of non-functional protein aggregates [34]. By binding to exposed hydrophobic regions of these proteins, chaperones shield them from inappropriate interactions and guide them along the correct folding pathways.

HSPs are a well-known class of chaperones that play a key role in cellular stress responses. Among them, HSP70 and HSP90 have particular prominence [35]. HSP70, also known as the 70-kDa heat shock protein, assists in the folding of newly synthesized proteins and promotes the refolding of denatured or misfolded proteins. This is accomplished by

transiently binding to exposed hydrophobic regions of unfolded proteins, preventing their aggregation and facilitating the correct folding process [34]. HSP90, on the other hand, is involved in the stabilization and activation of a wide range of client proteins, including signaling molecules and transcription factors [36].

The assistance provided by chaperones is not limited to the cytoplasm, they are also involved in processes occurring in cellular organelles such as the endoplasmic reticulum (ER) and mitochondria. In the ER, chaperones aid in the folding of secretory and membrane proteins [37]. Additionally, chaperones help in transporting proteins across membranes and targeting them to specific cellular compartments [38]. Overall, the role of chaperone proteins is essential for maintaining cellular proteostasis, ensuring that proteins are correctly folded and functional, and preventing the deleterious effects of protein misfolding and aggregation. Understanding the intricate mechanisms of chaperone function provides valuable insights into cellular physiology and has implications for various diseases associated with protein misfolding, including neurodegenerative disorders and cancer [39, 40].

3.1.2 Role of folding enzymes

Protein folding is a highly orchestrated and complex process, and disulfide bond formation represents a critical step in this intricate dance of molecular interactions. Enzymes play a fundamental role in the intricate process of protein folding, ensuring the proper three-dimensional structure. Among these enzymes, protein disulfide isomerases (PDIs) play a crucial role in catalyzing the formation and rearrangement of disulfide bonds [41]. Disulfide bonds are covalent linkages between two cysteine residues in a protein, and their correct formation is pivotal for stabilizing protein structures [42]. PDIs assist in the correct pairing of cysteine residues by catalyzing the exchange and rearrangement of disulfide bonds, both within a single polypeptide chain (intra-molecular) and between different chains (intermolecular) [41].

The importance of PDIs extends beyond merely facilitating disulfide bond formation. These enzymes also play a role in preventing the formation of incorrect disulfide linkages, which could lead to misfolded or non-functional proteins. Additionally, PDIs contribute to the quality control mechanisms within the ER, where many proteins are synthesized and folded. By ensuring the fidelity of disulfide bond formation, PDIs help maintain the integrity of the cellular proteome [41]. The role of folding enzymes, particularly PDIs, goes beyond catalyzing the formation and rearrangement of disulfide bonds. These enzymes are integral to the precise and controlled folding of proteins, influencing their structural stability, proper function, and ultimately, their contribution to cellular processes.

3.1.3 Role of quality control systems

Cells have evolved intricate quality control systems to maintain the fidelity of the proteome by recognizing and selectively eliminating misfolded or damaged proteins [32]. These quality control mechanisms are crucial for cellular function and are designed to prevent the accumulation of aberrant proteins that could compromise cellular integrity [43]. One major pathway involved in targeted protein degradation is the ubiquitin–proteasome system. This highly regulated system relies on the attachment of ubiquitin molecules to misfolded or damaged proteins, marking them for recognition and subsequent degradation by the proteasome [44]. The proteasome, which acts as a cellular "recycling center," breaks down tagged proteins into smaller peptides that can be reused for new protein synthesis.

Despite the effectiveness of quality control mechanisms, misfolded proteins can sometimes evade these systems, leading to their accumulation within the cell. This can be attributed to various factors such as genetic mutations, environmental stressors, or imbalances in protein production and folding capacity [45]. The overwhelmed cellular defense systems may thereby result in the persistence of misfolded proteins, posing a serious threat to cellular function. Such accumulation of misfolded proteins is particularly implicated in the pathogenesis of various diseases, including neurodegenerative disorders and cancer [39, 40].

Neurodegenerative disorders, characterized by the progressive loss of structure and function of neurons, often involve the aggregation of misfolded proteins. These aggregates can disrupt normal cellular processes and contribute to the degeneration of neuronal tissues [39]. In the context of cancer, the cellular microenvironment surrounding malignant cells plays a crucial role in protein misfolding [12]. Tumor cells frequently experience adverse conditions such as hypoxia, nutrient deprivation, and oxidative stress, all of which can perturb the proteostasis network [46]. Disruption of protein homeostasis in cancer cells may eventually contribute to their survival and growth, highlighting the intricate interplay between cellular stress and protein quality control mechanisms.

3.2 Imbalance in proteostasis and tumor progression

Understanding the role of imbalance in proteostasis and accumulation of misfolded proteins in tumorigenesis and tumor progression is essential for developing targeted therapies that aim to modulate protein folding and maintain proteostasis in cancer cells. There are few cellular processes that connect the imbalance in proteostasis with tumorigenicity and tumor progression, as discussed below.

3.2.1 Role of protein aggregation

Protein aggregation represents a significant challenge to cellular proteostasis, as misfolded proteins can assemble into larger structures known as aggregates. These aggregates can have detrimental effects on cellular function and contribute to cellular toxicity. The process of protein aggregation often involves the formation of inclusion bodies, which are dense, insoluble structures containing aggregated proteins. Inclusion bodies can disrupt cellular processes and interfere with the normal functioning of organelles, ultimately compromising the health and viability of the cell [47].

A widely studied example of protein aggregation is observed in neurodegenerative disorders, where proteins, such as amyloid-beta in Alzheimer's disease, alpha-synuclein in Parkinson's disease, and huntingtin in Huntington's disease, aggregate and form inclusion bodies [48–50]. The presence of these aggregates is closely linked to the pathology of these diseases, as they can induce cellular stress, trigger inflammatory responses, and lead to neuronal dysfunction and death. Therefore, any imbalance in proteostasis, characterized by an accumulation of misfolded proteins and formation of aggregates, commonly observed in neurodegenerative disorders, highlight the importance of understanding the mechanisms underlying protein aggregation [51].

In the context of cancer, the role of protein aggregation in tumor progression is an active area of investigation [52–54]. Various studies have identified the existence of different misfolded protein aggregates in different cancer cell types which are listed in Table 1. The tumor microenvironment, characterized by factors such as hypoxia, nutrient deprivation, and oxidative stress, can contribute to the misfolding of proteins and the subsequent formation of aggregates [12]. The accumulation of protein aggregates in cancer cells may affect crucial cellular processes, including cell cycle regulation, DNA repair mechanisms, and signaling pathways, influencing the overall behavior of cancer cells.

3.2.2 Role of dysregulation of autophagy

Autophagy plays a pivotal role in maintaining cellular homeostasis through targeted degradation and recycling of damaged or unnecessary cellular components. This intricate process involves the formation of autophagosomes, double-membraned vesicles that engulf cytoplasmic cargo, including misfolded proteins, and deliver them to lysosomes for degradation [67]. Dysregulation of autophagy has been implicated in various diseases, including cancer, where its dual role in both promoting and suppressing tumorigenesis is being increasingly recognized [68]. Autophagic dysregulation in cancer involves accumulation of misfolded proteins and damaged organelles within the cell [69]. Normally, autophagy acts as a quality control mechanism by removing potentially harmful cellular components and preventing the buildup of cellular stress. However, when autophagy is impaired, as seen in certain cancer cells, there is an increased risk of genomic instability and the activation of pro-survival pathways that facilitate tumor progression [68]. The accumulation

Table 1 Different misfolded protein aggregates in different cancers

Proteins that are misfolded	Type of cancer	References
P53	Neuroblastoma, breast cancer, lung cancer, oral cancer, stomach cancer	[55–59]
NCoR	Promyelocytic and monocytic acute myeloid leukemia, Non-small cell lung cancer	[60, 61]
BRCA1	Breast cancer	[62]
Ectopic clotting factor VIII	Hepatocellular carcinoma	[63]
Immunoglobulin	Myeloma	[64]
Alpha-synuclein	Melanoma	[65]
Tau	Prostate	[66]

of misfolded proteins, in particular, can disrupt cellular functions and contribute to the genomic alterations that underlie the initiation and progression of cancer [70].

However, in some contexts, autophagy can paradoxically promote tumor cell survival. Cancer cells often experience periods of nutrient deprivation and metabolic stress as they rapidly proliferate. Under such conditions, autophagy can be exploited by cancer cells to reserve nutrients and energy through the degradation of cellular components. This adaptive response enables cancer cells to withstand the challenging microenvironment within tumors and support their uncontrolled growth [67]. Thus, understanding the intricate balance of autophagy in cancer is crucial for developing targeted therapies that either enhance or inhibit autophagic processes based on the specific needs of the tumor microenvironment [69].

3.2.3 Role of ER stress

The ER is a central organelle responsible for the synthesis, folding, and modification of proteins destined for various cellular compartments. Proper protein folding is essential for maintaining cellular homeostasis, and the ER has a sophisticated quality control system to ensure the correct folding of nascent proteins [71]. When the folding capacity of the ER is overwhelmed due to factors such as increased protein synthesis, alterations in calcium homeostasis or oxidative stress, a condition known as ER stress ensues. ER stress eventually triggers the unfolded protein response (UPR), a cellular signaling pathway aimed at restoring protein homeostasis within the ER [72].

Persistent or unresolved ER stress can lead to the accumulation of misfolded and unfolded proteins in the ER lumen, forming aggregates that can be toxic to the cell. This condition is particularly relevant in the context of cancer, where cells often experience heightened demands for protein synthesis and folding to support their rapid proliferation [46]. The accumulation of misfolded proteins due to prolonged ER stress can activate pro-apoptotic pathways, leading to cell death [73]. In some instances, however, cancer cells exploit the UPR to enhance their survival by promoting adaptive responses that alleviate ER stress, allowing continued cell growth and resistance to adverse conditions [74].

The role of ER stress in cancer extends beyond the mere accumulation of misfolded proteins. ER stress-induced signaling cascades can impact various cellular processes, including inflammation, metabolism, and immune responses, all of which contribute to the complex microenvironment of tumors [46]. Additionally, UPR can influence the behavior of cancer cells by modulating key signaling pathways involved in cell survival and proliferation [75]. Understanding the interplay between ER stress and cancer progression is crucial for identifying potential therapeutic targets. Researchers are exploring strategies to selectively induce or inhibit UPR in cancer cells to either enhance their vulnerability to ER stress-induced apoptosis or disrupt adaptive responses that support tumor growth [76].

3.2.4 Role of UPR

Cells activate the UPR as a protective response to cope with ER stress. However, chronic activation of the UPR can contribute to tumorigenesis. UPR can influence cell survival, apoptosis, and inflammation through some specific signaling pathways, discussed in more detail below (Sect. 3.4), providing evidence for its role in tumor microenvironment and promoting tumor progression [77].

3.2.5 Role of mutations and genetic instability

The accumulation of misfolded proteins within cells, often associated with proteostasis imbalance and cellular stress, has far-reaching consequences that extend beyond the immediate cellular milieu. One profound effect is the potential to induce genetic instability, a hallmark of cancer development and progression [78]. Genetic instability refers to an increased propensity for alterations in the DNA sequence, which can manifest as mutations, chromosomal rearrangements, and aneuploidy. These genetic aberrations, particularly mutations, can disrupt the normal functioning of critical genes involved in fundamental cellular processes [79].

The link between misfolded proteins, cellular stress, and genetic instability is particularly significant in cancer. The accumulation of misfolded proteins may activate signaling pathways that contribute to genomic alterations. Cellular stress responses, including those induced by the UPR in the ER, can influence the activity of key genes involved in cell cycle regulation, DNA repair, and apoptosis [46]. Mutations in these genes can disrupt the carefully orchestrated mechanisms that ensure genomic integrity, potentially leading to uncontrolled cell growth and tumor formation.

Understanding the molecular basis of protein folding and the consequences of misfolding is essential for devising strategies to intervene in diseases associated with proteostasis imbalance, including cancer. Researchers are therefore actively exploring ways to—(i) enhance cellular protein-folding capacity, (ii) promote the efficient clearance of misfolded proteins, and (iii) modulate cellular stress responses to mitigate the impact of protein misfolding on cell function and health [80]. Strategies aimed at preventing or repairing genetic alterations resulting from misfolded protein-induced stress hold promise for the development of novel therapeutic interventions in cancer and other diseases associated with protein misfolding and cellular stress. Such targeted approaches may offer new avenues for precision medicine, allowing for more effective and less toxic treatments tailored to the specific molecular characteristics of individual tumors.

3.3 Proteostatic stress in CSCs

CSCs represent a distinctive subset within tumors, wielding unique properties such as self-renewal and multi-lineage differentiation, which significantly contribute to tumor initiation, progression, and therapeutic resistance [81, 82]. The delicate balance that CSCs must strike between maintaining their self-renewal capacity and managing misfolded proteins places an unprecedented demand on their protein quality control systems [10]. This demand is further compounded by the heterogeneity inherent in CSC populations. An additional critical aspect of the CSCs is the dynamic equilibrium between self-renewal and differentiation potential [83]. CSCs possess the ability to generate identical CSCs through self-renewal and also differentiate into diverse cell types within the tumor. Achieving this balance necessitates precise protein quality control to ensure correct folding and functionality of proteins crucial for both processes [8]. This intricate regulation is essential for perpetuation of CSC characteristics.

Moreover, CSCs play a pivotal role in tumor initiation, progression and even recurrence, contributing to heterogeneity within the tumor and dissemination to other organs [84]. The protein quality control mechanisms must meticulously oversee the proper folding and function of proteins involved in signaling pathways, cell cycle regulation, and other processes essential for tumor growth. This complexity extends to therapeutic resistance, as CSCs are often implicated in evading conventional cancer therapies [85]. Protein quality control systems in CSCs thereby contribute to therapy resistance by ensuring CSC survival under stressful conditions and maintaining the functionality of resistance-associated proteins.

Perturbations in the activity of protein homeostasis within CSCs are of considerable interest due to their potential association with the pathobiology of these cells and therapeutic opportunities. A study employing glioblastoma and breast carcinoma cells, genetically modified to express a proteasome substrate fused with green fluorescent protein for in vivo tracking, demonstrated that CSCs exhibited diminished proteasome activity compared to cells grown in monolayers [86]. A modification of the transfection construct involved the incorporation of a thymidine kinase sequence, facilitating the pharmacological targeting of cells characterized by low proteasome activity, and allowing for the stable expression of the construct. Treatment with ganciclovir, activated by co-expressed thymidine kinase, led to the efficient regression of xenotransplants derived from breast cancer cell lines T47D and MDA-MB-231, which were specifically sorted for low proteasome activity in mice [87].

Another investigation, utilizing the same proteasome substrate fused to a green fluorescent protein system in transfected osteosarcoma cell lines, revealed that cells exhibiting low proteasome activity (positive for green fluorescence) underwent asymmetric division, generating cells with either high or low proteasome activity. In contrast, cells with higher proteasome activity (green fluorescence negative) produced progeny exclusively negative for green fluorescence. Additionally, the study confirmed that cells with low proteasome activity displayed a heightened capacity for sphere formation compared to their counterparts with high proteasome activity [88]. Non-small cell lung carcinoma cell lines in sphere cultures also exhibited lower proteasome activity and enhanced tumorigenicity in mice [89]. Additionally, prostate cancer cell fractions with low proteasome activity displayed increased radioresistance [90]. Orthotopic injection of breast cancer cells with low proteasome activity in mice produced larger tumors and more metastasis than their high proteasome activity counterparts [91].

Human glioblastoma cells with low proteasome activity were more tumorigenic in mice, and expressed higher levels of the stem cell marker, Sox2 [92]. Another study revealed that radiation of human head and neck cancer cell lines resulted in an increased proportion of cells exhibiting reduced proteasome activity. This observation aligns with the hypothesis that CSCs with low proteosomal activity play a pivotal role in conferring radioresistance to tumors [93]. Colon cancer cell fractions with stem cell properties, including increased sphere formation capacity and resistance to chemo- and radiotherapy, also displayed low proteasome activity [94]. Inhibition of specific proteasome enzymatic activities in breast cancer cells increased the expression of CD44, a stem cell marker, and promoted epithelial-mesenchymal transition. Moreover, patients with breast cancer expressing low levels of specific proteasome units exhibited worse survival outcomes [95].

The metabolic demands of CSCs, characterized by altered energy metabolism, further influence the protein quality control landscape. The complex metabolic activity of CSCs demands for proper protein folding and degradation. Disruptions in proteostasis may impact CSC survival and function, adding an additional layer of intricacy to their protein quality control systems [83, 96]. Furthermore, CSCs exhibit inherent heterogeneity, with individual cells displaying varying degrees of self-renewal and differentiation potential [97]. The protein quality control systems must adapt to this diversity, addressing the proteomic landscape associated with different CSC states. The adaptability of these quality control mechanisms is paramount for maintaining cellular homeostasis within the dynamic context of CSCs, where stability and balance must be preserved despite the inherent variability in CSC characteristics. In essence, the effectiveness of protein quality control systems is crucial in influencing the behavior of CSCs, playing a central role in their diverse states and contributing to the overall complexity of cancer biology.

3.4 Clearance of misfolded proteins from CSCs

Within the cellular quality control system, the clearance of misfolded proteins is orchestrated through highly regulated mechanisms, prominently involving the proteasomal degradation and autophagy pathways [98]. A recently discovered mechanism in the intricate landscape of protein homeostasis maintenance within cancer cells also focuses on exosomal clearance of misfolded proteins, as depicted in Fig. 1. Research findings indicate that the regulation of proteostasis through exosomes involves a coordinated interplay of both proteasomal degradation of impaired proteins [99] and the process of autophagy [100].

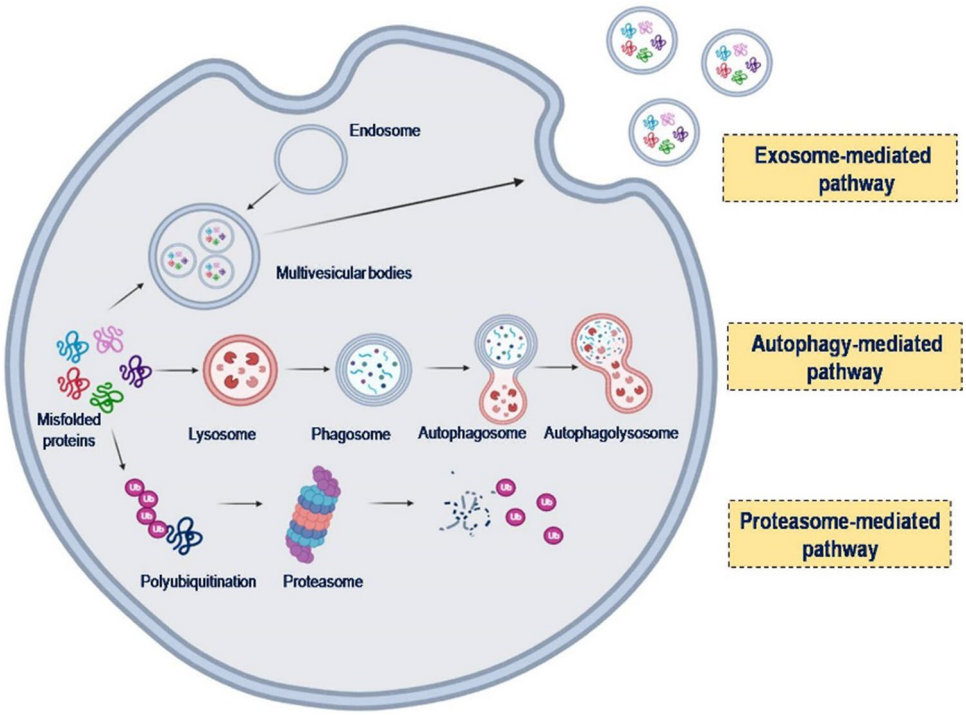

Fig. 1 Clearance of misfolded proteins from CSCs may occur through proteasome-, autophagy- or exosome-mediated pathway. Proteasome-mediated degradation involves the ubiquitin–proteasome system (UPS), where proteins tagged with ubiquitin are recognized and processed by the 26S proteasome, leading to their degradation into small peptides. This pathway is essential for protein quality control and cellular homeostasis in CSCs. Autophagy is a lysosome-dependent degradation pathway that handles the clearance of larger protein aggregates and damaged organelles. In macroautophagy, misfolded proteins are sequestered into autophagosomes, which then fuse with lysosomes to form autolysosomes, where the contents are degraded by lysosomal hydrolases. Autophagy supports the metabolic flexibility and survival of CSCs under stress conditions. Exosome-mediated secretion is a novel pathway for the clearance of misfolded proteins. Exosomes are small extracellular vesicles that originate from the endosomal system. Multivesicular bodies (MVBs) containing intraluminal vesicles (ILVs) release exosomes upon fusion with the plasma membrane. Exosomes can encapsulate misfolded proteins and carry them away from CSCs, reducing proteotoxic stress. Once released, exosomes can be taken up by recipient cells in the tumor microenvironment, potentially influencing their function and contributing to tumor progression. Crosstalk between these pathways ensures a coordinated response to maintain protein homeostasis and support the stemness and survival of CSCs

3.4.1 Proteasome-mediated pathway

The proteasome, a large multi-subunit protein complex, is a central player in the ubiquitin–proteasome system responsible for targeted protein degradation. In this process, proteins marked for disposal are tagged with ubiquitin molecules, guiding them to the proteasome for degradation. The proteasome then unfolds and translocates the substrate protein into its catalytic core, where it is cleaved into smaller peptides. This controlled degradation of ubiquitinated proteins by the proteasome is fundamental to maintaining cellular protein homeostasis [101].

3.4.2 Autophagy-mediated pathway

Complementing the proteasomal degradation pathway, autophagy serves as another critical mechanism for the removal of misfolded proteins and cellular components [102]. Autophagy involves the formation of double-membraned structures known as autophagosomes, which engulf cytoplasmic cargo, including misfolded proteins, organelles and other cellular constituents. The autophagosomes subsequently fuse with lysosomes, forming autophagolysosomes, where the engulfed cargo is degraded by lysosomal enzymes. This process provides a bulk degradation mechanism, ensuring the removal of damaged or surplus cellular components. Autophagy thereby plays a crucial role in cellular adaptation to stress conditions and contributes to maintaining cellular homeostasis [59]. Deregulation of the proteasomal degradation and autophagy pathways is frequently associated with various diseases, including cancer [102]. Impaired proteasomal activity can lead to the accumulation of misfolded proteins and dysregulation of critical cellular processes, contributing to tumorigenesis [8]. In addition, impaired autophagy may lead to the accumulation of damaged organelles and proteins, promoting genomic instability and tumorigenesis [103]. The intricate balance between these degradation pathways is vital for preventing the build-up of aberrant proteins that could compromise cellular function and genomic stability.

Exosomes, which are small extracellular vesicles released by cells, act as specialized carriers orchestrated by cancer cells for the disposal of aberrantly folded proteins. A comparable interplay is anticipated within CSCs due to their imperative need for a finely tuned equilibrium during proteostasis. Research conducted on breast CSCs and prostate CSCs has revealed a robust association between autophagy and exosomes [104]. This mechanism could be a pivotal component in preventing proteotoxic stress, a state in which an excess of misfolded proteins poses threat to cellular function, as represented in Fig. 2. The selective packaging of specific misfolded proteins into exosomes adds an intriguing layer of complexity to the cellular quality control system, suggesting a level of precision in the disposal of proteins detrimental to CSC function.

4 Cancer stem cell-derived exosomes: a key player in tumor progression

4.1 Advent of exosomes

Exosomes have captivated researchers in recent years due to their role in intercellular communication. Initially discovered in the 1980s during the study of reticulocyte maturation [105, 106], exosomes were thought to be primarily involved in shedding cellular debris. Studies hinted at their potential involvement in clearing unwanted cellular components, such as damaged and obsolete proteins [107]. This idea gained validation in neurodegenerative diseases, where exosomes were implicated in removing toxic protein aggregates associated with conditions like Alzheimer's [108] and Parkinson's [109].

However, as research progressed in the early 2000s, their significance broadened. Exosomes were found to transport various biomolecules, including proteins [110] and RNA [111], and exhibited profound influence on the function of recipient cells. In the latter part of the 2010s and throughout the 2020s, contemporary investigations into exosomes have predominantly centered on their targeted cargo delivery capabilities [112]. However, there remains a substantial realm to be explored concerning the potential of exosomes as agents for clearance processes. Investigations focus on understanding the mechanisms by which exosomes facilitate the removal of cellular waste, offering potential therapeutic applications [113].

4.2 Role of exosome in CSC-mediated tumor progression

With specific reference to CSCs, exosomes emerged as essential contributors to the dynamic interplay between cancer cells and their surroundings [114]. Various research findings have already established the diverse contributions of

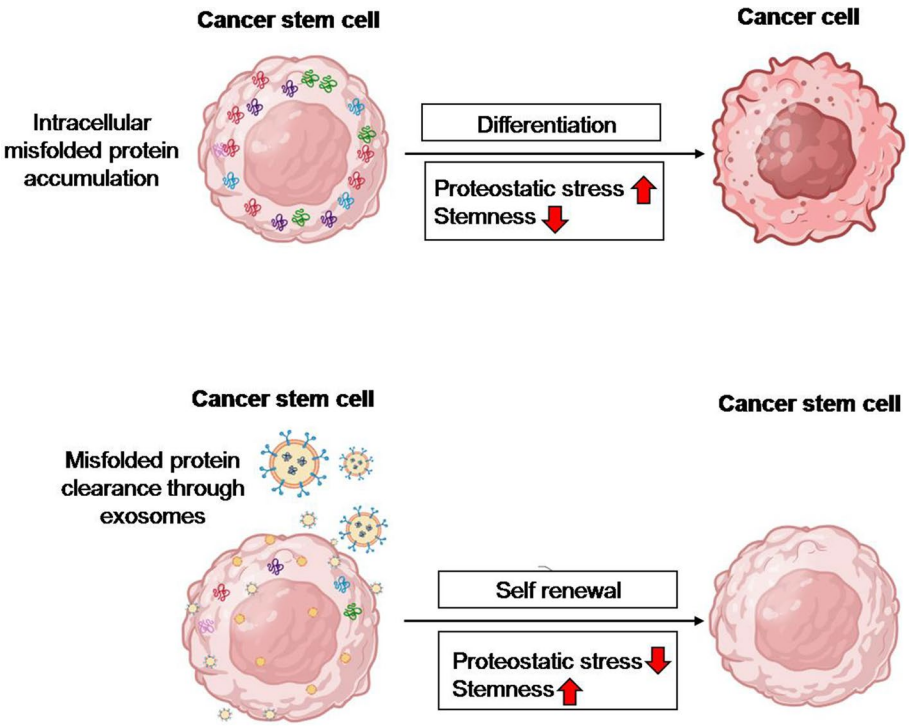

Fig. 2 Excess misfolded protein clearance through exosomes helps preventing proteostatic stress in cancer stem cells. **a** Accumulation of misfolded proteins within CSCs leads to proteostatic stress, triggering the unfolded protein response (UPR) and promoting differentiation into non-stem cancer cells. This results in reduced stemness and increased proteostatic stress, as indicated by the respective arrows. **b** In contrast, CSCs can alleviate proteostatic stress by packaging misfolded proteins into exosomes, which are then secreted out of the cells. This exosome-mediated clearance reduces the burden of misfolded proteins, thereby supporting CSC maintenance and self-renewal, depicted by increased stemness and decreased proteostatic stress. The effective management of proteostatic stress via exosome secretion is crucial for maintaining CSC characteristics, highlighting the importance of this pathway in sustaining CSC populations and their role in tumor progression and therapy resistance

CSC-derived exosomes in cancer progression (Table 2). The cargo of exosomes is diverse, including proteins, nucleic acids (such as RNA and DNA fragments), lipids, and diverse signaling molecules. This rich repertoire enables exosomes to serve as vehicles for the exchange of molecular information, influencing cellular behaviors and responses [115].

Exosomes derived from CSCs orchestrate critical processes associated with cancer progression within the tumor microenvironment. These processes include the promotion of tumor growth, immune evasion, and establishment of

Table 2 Role of CSC-derived exosomes in cancer progression

Type of cancer stem cells	Functions	References
Lung CSCs	Promote pro-metastatic phenotypes	[116]
Renal CSCs	Induce pro-tumorigenic phenotype in mesenchymal stromal cells	[117]
Glioblastoma stem cells	Promote angiogenesis	[118]
Colorectal CSCs	Promote tumorigenesis and immune-suppressed tumor microenvironment	[119]
Liver CSCs	Promote invasion and angiogenesis	[120]
Pancreatic CSCs	Enhance drug resistance	[121]
Breast CSCs	Promote chemoresistance	[122]
Thyroid CSCs	Promote EMT	[123]
Gastric CSCs	Promote metastasis	[124]
Glioblastoma SCs	Enhance stemness and tumorigenicity of glioma cells	[125]
Brain tumor-initiating cells	Suppress T cell activity	[126]
Esophageal squamous cell carcinoma SCs	Facilitate the maintenance of cancer stem-like cell dynamics equilibrium	[127]

the pre-metastatic niche [128]. Exosomes play a role in modulating immune responses by carrying immunosuppressive molecules and promoting an immunosuppressive microenvironment, thereby facilitating tumor immune evasion [129]. Additionally, they contribute to the formation of pre-metastatic niches by preparing distant organs for incoming cancer cells [130]. In the complex network of cellular communication, exosomes derived from CSCs stand out as pivotal regulators of the tumor microenvironment, influencing both local and systemic aspects of cancer progression.

Notably, the cargo of exosomes derived from CSCs contains specific molecules associated with stemness and therapy resistance. These molecules include stem cell markers, regulatory factors involved in self-renewal, and components associated with resistance to conventional cancer therapies [131]. The presence of such molecules in exosomes underscores their significance in mediating CSC-related processes and suggests a role in the dissemination of tumorigenic traits to neighboring and distant cells [132]. Understanding the intricate molecular cargo of CSC-derived exosomes will provide valuable insights into the functional consequences of intercellular communication mediated by these vesicles and open avenues for exploring targeted therapeutic interventions to disrupt these processes.

Exosomes particularly derived from CSCs also play a pivotal role in shaping the tumor microenvironment [133]. Their diverse cargo facilitates the exchange of molecular information between cells, influencing various aspects of cancer biology [134]. There have been reports proposing exosome-mediated elimination of misfolded proteins from cancer cells [135]. This analogous mechanism in CSCs may represent a novel aspect of CSC biology, providing insights into additional dimensions of how these cells intricately regulate protein homeostasis. Previous research has demonstrated that exosomes play a role in the targeted delivery of toxic proteins in neurodegenerative diseases [136] and in the clearance of misfolded proteins in the context of cancer [137]. Similar and underexplored functions of exosomes released by CSCs may involve serving as vehicles for targeted disposal of proteins that have the potential to compromise cellular integrity. This process may not only help in maintaining the functional integrity of CSCs, but also contribute to their survival under conditions that induce proteotoxic stress. The molecular specificity of exosomal clearance therefore raises questions about the criteria for selecting misfolded proteins and the regulatory mechanisms governing their packaging into exosomes.

Understanding the interplay between the newly-discovered exosomal mechanism and traditional protein clearance pathways is essential for obtaining a more comprehensive view of the regulatory networks governing proteostasis in cancer. The crosstalk between exosomal clearance and other cellular quality control systems, such as proteasomal degradation and autophagy, is likely to be intricate and context-dependent. Investigating these interactions may reveal how CSCs integrate multiple pathways to finely tune their protein quality control responses. This knowledge may hold promise for therapeutic interventions targeting protein homeostasis in cancer, with potential applications in disrupting the adaptive mechanisms that contribute to CSC survival and therapy resistance. Further exploration of the molecular details surrounding exosomal clearance within CSCs is warranted, as it may uncover novel biomarkers or therapeutic targets for precision cancer medicine.

4.3 Exosomal misfolded proteins in tumor progression

Protein misfolding can occur during various biochemical processes and is linked to the development of diseases, including cancer, characterized by genetic instability. In cancer, the microenvironment surrounding malignant cells exposes them to stressful conditions that may further promote protein misfolding, contributing to the complex landscape of tumorigenesis [12]. The genesis of tumor development frequently arises from mutations that disrupt the customary functions of pivotal regulatory proteins, including tumor-suppressor proteins and oncogenes. Such mutations can lead to modifications in the catalytic activity, the loss of binding sites for effector proteins, or alterations in the native folded conformation of the proteins [137].

The role of exosomal misfolded proteins in tumor progression presents a contradictory phenomenon [135]. While exosomes released by CSCs can act as carriers for the disposal of misfolded proteins, their internalization by neighboring cells can induce a cascade of events that paradoxically promotes tumor progression. Understanding this dual nature of exosomal misfolded proteins will provide critical insights into the complex dynamics of cancer progression. The cargo of exosomes, including misfolded proteins, can influence the signaling landscape of the recipient cells, potentially activating pathways associated with cell survival, proliferation, and evasion of apoptosis. This signaling activation by CSC-derived exosomes may contribute to the acquisition of tumorigenic traits by recipient cells, fostering an environment conducive to tumor progression [133]. The specifics of the signaling events triggered by exosomal misfolded proteins are likely to be context-dependent, varying with the cellular and micro environmental factors present. However, it can be anticipated that upon internalization by neighboring cells, exosomes containing misfolded proteins may trigger ER stress and UPR which act as pro-tumorigenic signaling pathways [138].

Furthermore, exosomal proteins have been implicated in the establishment of a pre-metastatic niche [139]. The transfer of exosomes containing misfolded proteins to distant organs can add to creation of a niche and may help in creating a microenvironment that is favorable for the seeding and growth of disseminated cancer cells. These misfolded proteins may act as initiators of molecular cascades that prepare the pre-metastatic niche, modulating the extracellular matrix, and promoting angiogenesis, which are essential processes for colonization of metastatic cells. This diverse role of exosomal misfolded proteins, not only as inducers of pro-tumorigenic phenotypes but also as contributors to the formation of a pre-metastatic niche, underscores the complexity of their impact on cancer progression.

The intricate interplay between exosomal misfolded proteins and tumor progression highlights the need for a nuanced understanding of the molecular events occurring within the tumor microenvironment. As evidenced in Parkinson's and other neurodegenerative diseases [140], revealing the specific mechanisms by which exosomal misfolded proteins influence neighboring cells and contribute to tumor progression will designate exosomes as novel therapeutic targets. Strategies aimed at disrupting these pro-tumorigenic effects may offer innovative avenues for cancer treatment. Further investigations into the molecular details of exosome-mediated effects on tumorigenesis and tumor progression will advance our understanding of these complex interactions and ideate the development of specific targeted therapies in the evolving field of cancer biology.

4.4 Mode of action of exosomal misfolded proteins

Critical examination of the intricate molecular mechanisms that govern the dual functions of exosomal misfolded proteins, shedding light on their impact within the realm of cancer biology, was investigated. At the core of these mechanisms lies the exosomal cargo, encompassing misfolded proteins, which serves as a communicator influencing the recipient cell behavior. The cargo is selectively packaged into exosomes, ensuring its targeted delivery to the neighboring cells [141]. The exosomes serve as conduits for the transmission of misfolded proteins to nearby cells, thereby instigating a cascade of events that significantly impact tumor progression. Upon internalization, these misfolded proteins can initiate a cascade of signaling events within the recipient cells, ultimately modulating various aspects of cellular behavior. Mostly, internalization of these misfolded proteins triggers relocalization of ER resident protein Glucose-regulated protein 78 (GRP78) to the cytoplasm which induces ER stress through some specific signaling pathway which needs further investigation [142]. In response to ER stress, cells activate the UPR, a complex signaling network aimed at restoring ER homeostasis [143].

The UPR is a cellular stress response mechanism aimed at maintaining protein homeostasis in the ER. The UPR comprises three interconnected signaling pathways, viz., the inositol-requiring enzyme 1 (IRE1) pathway, the activating transcription factor 6 (ATF6) pathway, and the protein kinase R-like ER kinase (PERK) pathway [144]. When unfolded or misfolded proteins accumulate in the ER, these sensors become activated to alleviate the stress. GRP78, also known as BiP, serves as a master regulator and chaperone that normally binds to the luminal domains of IRE1, ATF6, and PERK, rendering them inactive. However, during ER stress, GRP78 dissociates from these sensors, allowing their activation. IRE1 splices the mRNA of X-box binding protein 1 (XBP1), leading to the production of an active transcription factor that regulates genes involved in protein folding and degradation [145]. Additionally, IRE1 can interact with TNF receptor-associated factor 2 (TRAF2) to activate the June-N terminal kinase (JNK) pathway, promoting cell survival and proliferation. ATF6, once activated and cleaved in the Golgi apparatus, translocates to the nucleus to upregulate genes encoding ER chaperones and components of the ERAD pathway. This helps the cell cope with the increased load of unfolded proteins. In addition, ATF6 can indirectly contribute to chemoresistance by enhancing the capacity of the cell to manage proteotoxic stress [146]. PERK phosphorylates eukaryotic initiation factor 2α (eIF2α), leading to a temporary reduction in global protein synthesis. This attenuation allows the cell to reduce the influx of new proteins into the stressed ER. However, the translation of activating transcription factor 4 (ATF4) is upregulated under these conditions. ATF4 activates genes involved in antioxidant responses, amino acid metabolism, and autophagy. Persistent activation of ATF4 can lead to the induction of CHOP, promoting apoptosis in severe stress conditions, but it can also enhance survival pathways under moderate stress [147]. Together, these pathways, with the pivotal involvement of GRP78, collectively orchestrate a multifaceted cellular response to ER stress, promoting cell survival and restoring protein-folding homeostasis [148].

The consequences of exosome-mediated misfolded protein transfer and subsequent ER stress activation are profound and extend across various aspects of tumor development [116]. One critical outcome is the promotion of cancer cell survival, as activated UPR facilitates adaptive mechanisms to cope with proteotoxic stress [149]. Additionally, the evasion of apoptosis is facilitated by sustained activation of ER stress and UPR [150]. Moreover, the impact extends to the evasion of the immune system, with ER stress and UPR contributing to the establishment of an immunosuppressive

microenvironment that shields cancer cells from immune surveillance [151]. This immunosuppression hinders the body's natural defense mechanisms from recognizing and eliminating cancer cells. Furthermore, misfolded protein-mediated ER stress and UPR activation play a pivotal role in fostering metastasis, the spread of cancer to distant organs [152]. This is achieved through mechanisms such as enhanced cell migration and invasion, which are crucial steps in the metastatic cascade.

Angiogenesis, the formation of new blood vessels, is another facet influenced by exosome-mediated ER stress [153]. The activation of pro-angiogenic pathways contributes to the development of a vascular network that supports the growing tumor, ensuring a sufficient supply of nutrients and oxygen. Additionally, the acquisition of chemoresistance, a major challenge in cancer treatment, is facilitated by the sustained activation of ER stress and UPR. These mechanisms enable cancer cells to adapt and survive in the presence of chemotherapeutic drugs, rendering conventional treatments less effective [154].

The sustained activation of the UPR contributes to chemoresistance through several mechanisms. ER stress can upregulate the expression of ATP-binding cassette (ABC) transporters which pump chemotherapeutic drugs out of cancer cells, reducing their intracellular concentrations and effectiveness. The UPR also enhances the DNA repair capacity of cancer cells, allowing them to survive the DNA damage induced by chemotherapy. This involves the upregulation of genes involved in the DNA damage response (DDR) and repair pathways. Chronic ER stress can shift the balance towards anti-apoptotic signaling. For instance, the IRE1-XBP1 pathway can activate pro-survival factors such as B-cell lymphoma 2 (Bcl2), while PERK-ATF4 signaling can induce the expression of growth arrest and DNA damage-inducible protein 34 (GADD34), which dephosphorylates eIF2α, restoring protein synthesis and promoting cell survival [155]. Furthermore, ER stress induces autophagy, a cellular degradation process that helps cancer cells survive under chemotherapeutic stress by removing damaged organelles and proteins and providing metabolic substrates for cell survival [156].

Interestingly, the transfer of CSC-derived exosomal misfolded proteins and the subsequent activation of ER stress and UPR are not expected to be confined to local interactions. As CSC-derived exosomes orchestrate a pro-tumorigenic microenvironment, conducive to tumor progression in distant organs and potential metastatic sites, they can also disseminate these signaling cues to cells situated at those future metastatic sites. In essence, the interplay between exosomal misfolded proteins, ER stress and UPR may represent a sophisticated network that fuels various aspects of tumorigenesis and contributes to the complexities of cancer progression (Fig. 3).

A comprehensive exploration of the molecular mechanisms via which exosomal misfolded proteins may exert dual functions in tumor progression, has been depicted in Fig. 4. By assessing their role in maintenance of protein homeostasis in CSCs and at the same time, their effects on cell survival, angiogenesis, immune evasion, and the establishment of a metastatic microenvironment, the review contributes to our understanding of the diverse roles played by exosomes in shaping the complex landscape of cancer progression. These insights hold promise for the development of targeted therapeutic interventions aimed at disrupting the pro-tumorigenic effects of these CSC-derived exosomes. Moreover, the review delves into the intricate crosstalk between exosomal misfolded proteins and the downstream signaling pathways implicated in cancer progression. The identification of key molecular players and their interactions within this network provides a foundation for potential therapeutic targets. Understanding how these exosomes modulate essential pathways sheds light on the underlying molecular events that drive tumorigenesis. This knowledge not only enhances our grasp of cancer biology but also opens avenues for the development of targeted drugs that can disrupt these specific pathways, offering a more precise and effective approach in the battle against cancer.

5 Therapeutic implications

The revelation of the dual functions of exosomal misfolded proteins has promising therapeutic implications in the realm of cancer treatment. Targeting the intricate processes associated with the release, uptake, or downstream effects of exosomal misfolded proteins may emerge as a novel strategy to disrupt key events in cancer progression as it is observed in the context of neurodegenerative diseases [157]. Inhibition of the release of exosomal misfolded proteins from CSCs could potentially curtail their impact on neighboring cells, limiting the pro-tumorigenic signals propagated through exosomes. Conversely, strategies aimed at impeding the uptake of these exosomes by recipient cells may hinder the transmission of molecular cues that drive cellular transformation and the establishment of a pro-metastatic microenvironment.

Furthermore, the identification of specific misfolded proteins within CSC-derived exosomes can serve as a potential biomarker for cancer progression. The use of misfolded proteins as biomarkers provides a novel and valuable therapeutic strategy for several reasons. Firstly, the non-invasive nature of studying exosomes eliminates the need for invasive

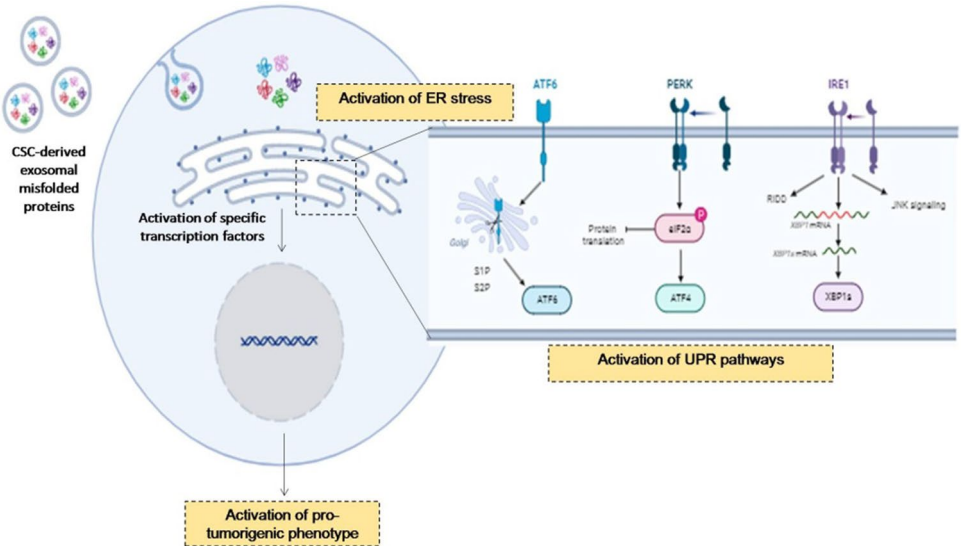

Fig. 3 The mode of action of CSC-derived exosomal misfolded proteins: inducing ER stress followed by activation of UPR pathways and ultimately contributing in activation of pro-tumorigenic phenotypes within the recipient cell. CSC-derived exosomal misfolded proteins induce ER stress and activate the UPR in recipient cells. Exosomes secreted by CSCs are internalized by neighboring cells, where the misfolded proteins accumulate in the cytoplasm. This leads to the relocalization of GRP78 (BiP) from the ER to the cytoplasm, triggering ER stress and UPR activation. The UPR involves three main pathways: ATF6, PERK, and IRE1. ATF6 translocates to the Golgi to release a transcription factor that upregulates ER chaperone and ER-associated degradation (ERAD) genes. PERK phosphorylates eIF2α, reducing global protein synthesis but increasing ATF4 translation, which regulates antioxidant and apoptosis-related genes. Under chronic stress, ATF4 can induce CHOP, a pro-apoptotic factor; however, CSC-derived exosomes may downregulate CHOP, reducing apoptosis and promoting survival. IRE1 splices XBP1 mRNA, producing XBP1s, which upregulates genes for protein folding and degradation, and activates JNK signaling via TRAF2, enhancing survival and proliferation. These pathways collectively promote a pro-tumorigenic phenotype by enhancing cell survival, proliferation, and stress resistance, highlighting the role of CSC-derived exosomal misfolded proteins in tumor progression

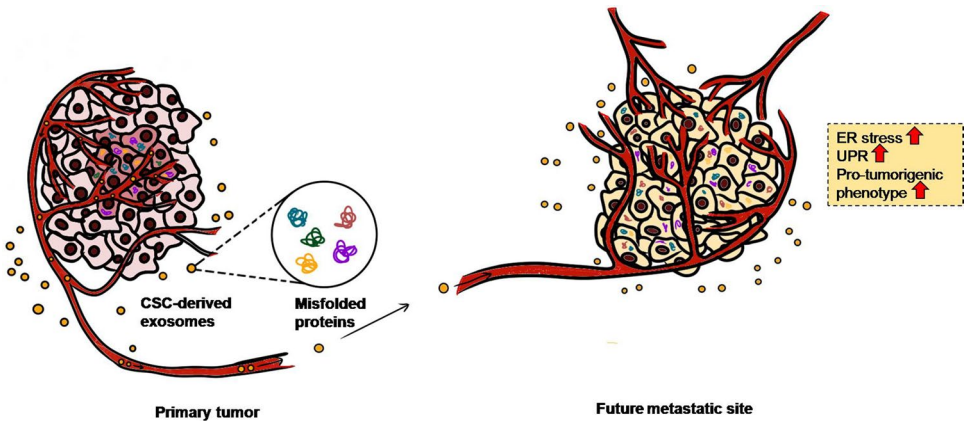

Fig. 4 Dual functionality of cancer stem cell-derived exosomes: maintaining proteostasis by clearing misfolded proteins from CSCs with concomitant delivery of misfolded proteins to future metastatic sites, instigating a pro-tumorigenic phenotype. Within the primary tumor, CSCs release exosomes containing misfolded proteins, which help alleviate proteostatic stress in the CSCs by exporting these potentially harmful proteins. This exosomal export mechanism maintains the protein homeostasis necessary for CSC self-renewal and survival. The released exosomes travel through the bloodstream and are taken up by cells at distant metastatic sites. Upon uptake by recipient cells, these exosomal misfolded proteins induce ER stress, triggering the UPR pathways. The UPR activation in recipient cells involves key pathways including ATF6, PERK, and IRE1, which collectively enhance the cell's capacity to manage misfolded proteins, but also inadvertently upregulate pro-survival and pro-tumorigenic signals. This stress response promotes the establishment of a supportive microenvironment for metastatic growth, characterized by increased ER stress, UPR activation, and a shift towards a pro-tumorigenic phenotype. Thus, CSC-derived exosomes play a critical role not only in maintaining CSC proteostasis but also in preparing distant sites for future metastasis by modulating cellular stress responses and enhancing tumorigenic potential

procedures to obtain tumor samples, making it more feasible for longitudinal studies and monitoring cancer progression over time. Secondly, misfolded proteins may play critical roles in signaling cascades and molecular pathways that drive cancer growth, making them attractive targets for therapeutic interventions.

Moving forward, future research efforts may delve into the development of advanced therapeutic modalities, such as nanotherapeutics, targeting exosomal misfolded proteins. In recent years, the PROteolysis TArgeting Chimeras (PROTAC) technology has surfaced as a highly promising strategy for eliminating specific disease-associated proteins, capitalizing on the intrinsic cellular machinery for protein degradation. Beyond PROTAC, various targeted protein degradation (TPD) methods, including molecular glue, Lysosome-Targeting Chimaera (LYTAC), and Antibody-based PROTAC (AbTAC), have also emerged [44, 158]. These versatile techniques hold significant potential for application in CSCs, aiming to degrade specific misfolded proteins. This dual action involves triggering proteasomal degradation, compelling the CSCs towards differentiation. Simultaneously, it intervenes in the dissemination of these exosomal misfolded proteins to distant organs via exosomes. Consequently, these innovative approaches offer a two-pronged strategy, not only compelling CSCs towards differentiation through heightened proteasomal degradation but also hindering the spread of detrimental CSC-derived exosomal misfolded proteins. Such interventions present novel avenues to curtail the deleterious effects associated with the presence of misfolded proteins in the context of cancer progression.

6 Future perspective

The specifics of CSC-derived exosomal misfolded proteins and their involvement in cancer progression remain an under-explored domain amongst current research strategies. There exists a pressing need for further investigation aimed at identifying specific exosomal misfolded proteins unique to various cancer types and revealing their molecular mechanisms in driving tumor progression. This avenue of research holds the potential to significantly augment our understanding of CSCs and their pivotal role in the intricate landscape of tumor progression. Moreover, the identification of specific misfolded proteins within CSC-derived exosomes stands to become a crucial focal point for therapeutic intervention in the battle against this formidable disease. Delving into the nuances of their molecular intricacies will not only enrich our knowledge base but also open up avenues for the development of targeted therapies, enhancing effective strategies for a more comprehensive cancer treatment and good patient prognosis in future.

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Author contributions U.C. conceptualized the review. A.B. wrote the main manuscript text and prepared the figures. All authors reviewed and approved the manuscript.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

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