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Review

Cancer Stem Cell Metastatic Checkpoints and Glycosylation Patterns: Implications for Therapeutic Strategies

Sara Sadat Aghamiri and Rada Amin *

Department of Biochemistry, University of Nebraska at Lincoln, Lincoln, NE 68503, USA
* Correspondence: raminali2@unl.edu or rada.aminali@gmail.com

Abstract: Cancer stem cells (CSCs), found within tumors, are powerful drivers of disease recurrence and metastasis. Their abilities to self-renew and maintain stem-like properties make treatment difficult, as their heterogeneity and metastatic properties can lead to resistance and limit the effectiveness of standard therapies. Given their significance, CSCs are typically isolated based on combinations of markers, which often indicate heterogeneous populations of CSCs. The lack of consensus in cell characterization poses challenges in defining and targeting these cells for effective therapeutic interventions. In this review, we suggest five promising molecules—ABCB5, CD26, CD66c, uPAR, and Trop-2—chosen specifically for their distinct distribution within cancer types and clinical relevance. These markers, expressed at the cell surface of CSCs, could significantly enhance the specificity of cancer stemness characterization. This review focuses on describing their pivotal roles as biomarker checkpoints for metastasis. Additionally, this review outlines existing literature on glycosylation modifications, which present intriguing epitopes aimed at modulating the stability and function of these markers. Finally, we summarize several promising in vivo and clinical trial approaches targeting the mentioned surface markers, offering potential solutions to overcome the therapeutic resistance of CSCs and addressing current gaps in treatment strategies.

Keywords: cancer stem cells; metastatic checkpoints; biomarkers; drug resistance; therapeutic resistance; clinical trials; targeted therapy

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1. Introduction

Cancer stem cells (CSCs), or tumor-initiating cells, are a heterogeneous subpopulation within the cancer bulk that exhibit stemness traits, such as self-renewal, pluripotency, and plasticity [1]. Their stemness properties enable them to transition between various states, adapting to environmental cues and rendering them more resilient to drug therapies. The intricate mechanism of stemness in cancer involves a complex network of signaling pathways, encompassing surface molecules, kinases, transcription factors, and epigenetic programming, which collectively shape their self-renewal and differentiation capabilities. For example, kinases like AKT, ERK, and mTOR regulate various cellular processes critical for CSC persistence, while transcription factors, such as OCT4, SOX2, and NANOG, orchestrate genetic programs associated with stemness. Additionally, surface markers such as CD44, and CD133 are widely used as markers to identify and isolate CSC populations across various tumor types [2]. These molecular components not only serve as biomarkers for identifying CSCs, but also present promising therapeutic targets for interventions to disrupt stemness maintenance and sensitize CSCs to conventional cancer treatments. Due to their properties, CSCs are considered critical drivers of tumor initiation, progression, and dissemination. The stemness phenotype has been identified in a variety of solid cancers and hematological cancers, indicating a conserved mechanism for tumor development and persistence [3–5]. While conventional regimens effectively target proliferative cancer bulk, CSCs have evolved multiple mechanisms to resist therapy pressure. These mechanisms include the modulation of surface proteins and their glycosylation patterns, with aberrant glycosylation being a hallmark of cancer [6].

In normal physiology, glycosylation is crucial for protein folding, cell–cell interactions, migration, signaling, and homeostasis [7]. Therefore, in the context of environmental communication, CSCs may upregulate specific surface markers associated with drug resistance mechanisms, metastasis, cell adhesion, and signaling pathways, promoting their survival and proliferation in the presence of therapeutic agents [8]. By modulating surface glycosylation, CSCs create a heterogeneous population of cells with diverse phenotypic characteristics, enabling them to adapt and survive therapeutic challenges. This versatility in surface marker expression significantly contributes to CSCs' therapy resistance, tumor recurrence, and metastasis [8].

While several surface markers of CSCs have been identified across multiple cancers, the same surface markers can also be found in normal stem cells [9]. The absence of consensus regarding phenotypic characterization specific to each cancer type, tissue, and infiltrated sub-clones at various sites has not yet been established. Therefore, there is a need to investigate unique markers or general surface markers for better molecular characterization to target them effectively. Nonetheless, targeting surface markers in CSCs holds significant promise in cancer therapy due to their unique properties [3]. Defining molecular checkpoints governing resistance and metastasis represents a promising therapeutic strategy with the potential to address the root cause of tumor growth, recurrence, and therapeutic resistance [10]. In this review, we propose five potential CSC metastatic checkpoints—ABCB5, CD26, CD66c, uPAR, and Trop-2—each characterized by surface-specific expression and cancer-type distribution. Selecting surface markers such as ABCB5, CD26, CD66c, uPAR, and Trop-2 stems from their emerging importance in CSCs and their potential clinical relevance. We prioritize these markers for their roles in cancer progression, metastasis, therapy resistance, and patient outcomes across various cancer types. Despite individual investigations into these markers, there is a need for comprehensive reviews synthesizing the existing literature, so as to understand their mechanisms and implications in cancer stemness. By reviewing the roles of these markers, we aim to identify areas for further investigation and pave the way for the development of novel cancer therapies targeting these key surface molecules. Hence, our narrative review underlines the pivotal role of these five glycoproteins in CSCs, as well as the significance of glycosylation in regulating their surface stability and function. We conclude by providing recent therapeutic strategies aimed at targeting these markers.

2. Key Metastatic Checkpoints as Biomarkers for Cancer Stem Cells

Cancer stem cells are characterized by the expression of specific surface glycoproteins, which orchestrate critical cellular processes involved in tumor initiation, progression, and metastasis. While numerous surface markers are common across various cancers, the quest for a consensus classification based on tissue and tumor type remains unfulfilled. Currently, CSCs are isolated based on marker combinations, often pointing to heterogeneous stem cell populations [11]. The widely shared surface markers among solid cancers are CD133 [12], CD44 [13], and EpCAM [14], while also commonly used for diagnosis and prognosis. Some surface glycoproteins that are cancer tissue-specific also exist (Table 1). In the following sections, we describe the significance of ABCB5, CD26, CD66c, uPAR, and TROP-2, which have been characterized as CSCs in specific cancer types and present their metastasis and chemoresistance properties.

Table 1. Summary of metastatic checkpoints based on CSC cancer types and associated stemness markers.

Marker Names	CSC Cancer Types	Associated Stemness Markers	References
ABCB5	Melanoma	CD133, CD44	[15,16]
	Liver cancer	CD133, EpCAM	[17]
	Glioblastoma	CD133	[18]
CD26/DPPIV	Colorectal cancer	CD133, CD44, EpCAM	[19–21]
CD66c/CEACAM6	Colorectal cancer	CD133	[22]
	Breast Cancer	CD44	[23]
uPAR/CD87	Small cell lung cancer	CD133, CD44	[24,25]
Trop-2	Prostate cancer	CD133	[26]

2.1. ABCB5

ATP-binding cassette subfamily B member 5 (ABCB5), a member of the ATP-binding cassette (ABC) transporter family, has emerged as a significant molecule in various biological processes in cancer. While most studies have identified ABC transporters' involvement in drug efflux and multidrug resistance (MDR), the characterization of ABCB5 in this context remains limited. However, it has been studied in physiologic conditions, where it regulates cell fusion in normal melanocytes [27–29]. In melanoma, CD133+/ABCB5+ cells are significantly more expressed in primary and metastatic melanomas, while CD44 is ubiquitous among ABCB5 positive and negative cells [15]. Using xenotransplantation, ABCB5-positive cells can re-initiate tumors and exhibit self-renewal and differentiation capacity. In addition, ABCB5 can generate both ABCB5+ and ABCB5− progeny, while ABCB5− tumor populations produce, albeit at lower rates, ABCB5− cells exclusively [15,16]. In liver cancer, the expression of the ATP transporter is observed alongside CD133 and EpCAM hepatic cancer stem cells. Genetic inhibition of ABCB5 in these cells sensitized them to chemotherapy, leading to apoptosis [17]. In glioblastoma, ABCB5 showed a positive correlation with CD133 expression in patients, and was linked to poor survival outcomes. The co-expression of ABCB5 with CD133 was confirmed through CSCs derived from cell lines. Furthermore, blocking ABCB5 in vivo halted tumor growth and increased tumor sensitivity to drug therapy [18].

Several studies have associated ABCB5 with CD133 or CD44, suggesting the potential of ABCB5 to be a marker of cancer stemness. For instance, in colon cancer, ABCB5 and CD133 are weakly co-expressed in rare cells within the normal human colon, while their levels are elevated in colorectal cancer. However, in this study, the authors did not confirm whether these cells were indeed CSCs. Intriguingly, ABCB5 shows high expression in less-differentiated colorectal cancer cells, which coincides with the presence of chemoresistant clones [30]. Although the in vitro knockdown of ABCB5 did not affect cell growth, the subsequent in vivo experiments revealed that these cells failed to develop tumor growth. Moreover, they exhibited increased sensitivity to therapy treatment, indicating a potential role for ABCB5 in tumor development and MDR [30]. Similarly, in oral squamous cell carcinoma (OSCC), ABCB5 exhibits weak expression in normal tissue but is markedly upregulated in OSCC. Although ABCB5 is expressed by CD44 cells in OSCC, the authors refrained from concluding that ABCB5 in OSCC represented CSCs due to the lack of in vitro and in vivo validation of the stem cell-like phenotype of these sub-clones [31]. Extensive characterization across various cancers has revealed that ABCB5 exhibits wide expression, spanning numerous cancer types, including breast, prostate, pancreas, brain, kidney, ovary, and others [32]. Its diverse expression in multiple cancers and its role in drug resistance highlights the importance of further investigating ABCB5's involvement in the chemoresistance properties of CSCs.

2.2. CD26

Dipeptidyl peptidase 4 (CD26), also known as DPPIV, serves as a pivotal peptidase involved in cleaving the N-terminus of regulatory peptides. While its expression is

widespread across various solid cancers, CD26 has emerged as a marker for CSCs, particularly in colorectal cancer. However, it is worth noting that CSCs in colorectal cancer appear to exhibit significant heterogeneity [21]. In this context, CD26-positive colorectal cancer (CRC) cells demonstrate elevated gene expression associated with stemness markers such as CD133, CD44, and CXCR4 [19]. Pang et al. found that the subpopulation CD26+ possesses stem-like features in primary and metastatic tumors in colorectal cancer patients with liver metastasis. In this study, the marker CD26 was differentially co-expressed with CD133 and CD44, showing the persistence of two main populations—CD133+, CD26+, CD44+ and CD133+, CD26+, CD44—among metastatic and non-metastatic cohorts [20]. These findings contrasted with another study conducted in CRC cell lines. The CD26 positive cells do not correlate with the main stemness markers such as CD133 and CD44, and the cell lines with sphere formation capacity englobe CD26+/CD133−. Instead, the authors found a greater association between CD26 and other CSC markers, such as E-cadherin, LGR5, and EpCAM in CRC cell lines [21]. Overall, CD26-positive CSCs in CRC mostly exhibit traits of invasiveness chemoresistance, and are often associated as a marker of distant metastasis [20,33]. Although CD26 was not identified as a CSC marker in pancreatic cancer, its metastatic properties have been noted in the disease [34]. Studies have shown that the deletion of CD26 leads to the inhibition of cell growth, invasion, and an increase in apoptosis, indicating its potential significance in pancreatic cancer progression [35]. CD26 exhibits multifaceted roles, including interactions with various components and involvement in diverse signaling pathways. The complexity of CD26 is highlighted by its numerous binding partners [36], implicating CD26 in various tumor processes, such as metastasis and progression. For instance, CD26 has been found to bind CXCR4, which is recognized as a marker for CSC in several cancer types, including breast [37], lung [38], brain [39,40], and colon cancer. Interestingly, CD26 expression correlates with CXCR4 expression in prostate cancer and has been associated with tumor stage and size [41]. Altogether, CD26 was shown to be an intriguing protein with multifaceted properties, indicating the worth of exploring more CD26 properties in CSCs across several cancers.

2.3. CD66c

Another potential stemness marker found in CRC is CEA cell adhesion molecule 6 (CEACAM6/CD66c), which belongs to the carcinoembryonic antigen family. CD66c is primarily involved in various cellular processes, including cell–cell adhesion, angiogenesis, migration, signal transduction, invasion, and metastasis formation [42]. Gemei et al. demonstrated that CD66c+/CD133+ cells represent colorectal tumor cells, whereas CD66c−/CD133+ is characteristic of adjacent normal tissue. In the same study, CD66c-positive cells showed the capacity to form spheroid in vitro and to induce metastasis in vivo. Notably, CD66c CSCs have the ability to generate CD66c negative cells, mirroring primary tumor cells in vivo, suggesting a plastic phenotype capable of generating subclones similar to the original tumor [22]. A comparative proteomic analysis of breast cancer cell lines identified CD66c as a novel stemness marker within CSCs-CD44 cell line cultures in vitro. However, the prolonged culture of CD44+/CD66c+ breast CSCs leads to a gradual decline in CD66c surface expression over time, accompanied by a notable decrease in CD44 expression. These findings suggest a phenotypic shift occurring over time in these cells [23]. Further investigation is necessary to assess and validate the expression of CD66c in breast CSCs patient samples. CD66c is also known to be overexpressed in certain cancers, particularly lung [43–45] and pancreatic cancer [46], where it contributes to tumor growth, invasion, and metastasis. However, the characterization of CD66c as CSCs in lung and pancreatic cancer has not yet been explored.

2.4. uPAR

The urokinase plasminogen activator surface receptor (uPAR), also known as CD87, serves as a receptor for urokinase plasminogen activator (uPA), a serine protease implicated in tissue remodeling, cell migration, and embryogenesis during physiological processes [47].

uPAR plays diverse roles in various pathological processes in cancer due to its proteolytic and non-proteolytic functions. Acting as a protease, the binding of uPA ligands to uPAR initiates the degradation of the extracellular matrix, thereby promoting metastasis [48]. Functioning as an adhesion receptor, uPAR can bind to the extracellular matrix, integrins, and G-protein-coupled chemotaxis receptors, thereby facilitating tumor progression, angiogenesis, and survival [49]. Furthermore, as a stem cell marker, uPAR promotes MDR in small-cell lung cancer (SCLC). Gutova et al. conducted in vitro screening of six SCLC cell lines, reporting the persistence of a small population of uPAR-positive cells (1–4%) among the CD44-positive cells. These uPAR-positive cells demonstrated spheroid formation and exhibited clear resistance to chemotherapy compared to their uPAR-negative counterparts. Notably, uPAR-positive cells were capable of forming colonies comprising both uPAR-positive and uPAR-negative cells, with proportions of 1–5% and 95–99%, respectively. This suggests that uPAR may confer the ability to revert to the parental cell phenotype [24]. Another study investigated uPAR both in vitro and in vivo across established SCLC cell lines from patients [25]. Kudo et al. selected CD133 as a putative cell-surface marker associated with uPAR. Both uPAR-negative and uPAR-positive cells exhibited resistance to chemotherapy. Similarly to the previous study from Gutova et al., CD133−/uPAR+ cells gave rise to both uPAR-positive and uPAR-negative sub-clones (CD133−/uPAR+ and CD133−/uPAR−), while double-negative cells generated only double-negative clones. Intriguingly, only double-negative clones were capable of initiating tumor growth in vivo compared to CD133−/uPAR+ cells. This discrepancy between in vitro and in vivo behaviors might be attributed to the higher quiescent population found among double-negative than uPAR single-positive populations (50% compared to 28%, respectively). Additionally, the discrepancy between the two studies might be influenced by the cell system used and the choice of stem cell marker, CD44, compared to CD133. Overall, these studies underscore the high heterogeneity of CSCs phenotype in lung cancer, emphasizing the need for consistency in phenotypic characterization and experimental setup. Additional investigations will be required to elucidate the potential role of uPAR as a cancer stem cell marker in lung cancer. Aberrant expression of uPAR and its ligand has been found in several invasive classes of cancers and often displays a poor prognosis, suggesting the aggressive cancer phenotype that uPAR might be orchestrating in those cancers [50]. Adding both metastatic and chemoresistance properties, uPAR can be part of an interesting biomarker to investigate across cancers.

2.5. Trop-2

The tumor-associated calcium signal transducer 2 (Trop-2), also known as trophoblast antigen 2, is a transmembrane glycoprotein highly expressed by various normal progenitor cells in tissues such as the liver [51], endometrium [52], and prostate [53]. It exhibits stemness properties like pluripotency, self-renewal, and tissue regeneration. Initially identified as a cell surface receptor involved in calcium signaling, intracellular signaling, cell adhesion, and migration [54], Trop-2 is now recognized as an oncogene due to its aberrant expression in numerous epithelial cancers, including breast, lung, prostate, ovarian, and colorectal cancers, among others. This makes it a potential biomarker for cancer diagnosis and prognosis [55,56]. Immunohistological studies have confirmed co-expression of Trop-2 with CD133 in malignant epithelial cells of the prostate [26], although the stemness properties of these clusters remain to be fully validated. To date, Trop-2 has only been identified as a cancer stem cell marker in prostate cancer in a single study. Its multifaceted role in cancer is evidenced by its association with poor survival outcomes, tumor progression, metastasis [54,57], and resistance to therapies [54]. Notably, Trop-2's expression in normal stem cells suggests its potential to initiate cancer if deregulated. Given its widespread expression in various cancers and its involvement in cancer stem-like phenotypes, Trop-2 emerges as an intriguing surface marker to explore as a therapeutic target for cancer treatment.

In summary, CD26, CD66c, and ABCB5 have been demonstrated separately in colorectal cancer. However, to this date, no study has investigated the three markers together in

the characterization of CSCs in colorectal cancer. On the other hand, surface markers, such as Trop-2 and uPAR, exhibit cancer-specific expression and have the potential to serve as CSC markers in other cancers.

3. The Glycosylation Signature of Metastatic Checkpoints

Glycosylation, a central mechanism in the post-translational modification process (PTMs), plays a crucial role in shaping the conformation and functionality of various molecules, such as proteins, lipids, and other organic compounds. Glycosylation is a complex process by which a glycan attaches to the amino acid residues of the protein backbone. Different types of glycan modifications are based on specific amino acid residues, including N-, O-, C-linked, and O-GlcNAc (N-Acetylglucosamine) modifications, with N-linked and O-linked being the most prevalent. In proteins, N-linked glycosylation involves the attachment of glycans to asparagine residues, while O-linked glycosylation involves the attachment of glycans to serine or threonine residues [7]. Given the importance of glycosylation in biological processes, cancer strategically employs glycosylation to modulate surface glycoprotein structure and function, influencing survival, immune evasion, adhesion, and even metastasis [58,59]. Aberrant glycosylation, a general characteristic feature of tumor cells, can lead to changes in both N-glycan and O-glycan structures, resulting in abnormal carbohydrate structures referred to as tumor-associated carbohydrates (TACAs). The specific types of glycans present in TACAs can vary depending on the cancer type and the glycosylation alterations occurring within the tumor cells [60]. Common alterations in cancer glycosylation include defects in glycan maturation, causing the expression of truncated glycan chains, as well as the expression of complex glycan motifs and loss of certain glycans [61]. Notably, TACAs can serve as biomarkers for cancer diagnosis, prognosis, and therapeutic targeting and are widely utilized in clinical diagnostics [60]. Therefore, targeting TACA, or glycan-binding proteins, represents a promising approach for CSCs therapy, offering the potential to target cancer cells while sparing normal tissues selectively [60]. In the following sections, we described the literature findings on the N- and O-glycosylation of these metastatic CSC markers.

3.1. ABCB5

The transporter ABCB5 comprises a total of 14 N-glycosylation sites across its protein core, two of which are situated within the extracellular loop region [62]. Despite the abundance of N-glycosylation sites, the N- and O-linked glycosylation of ABCB5 has not been as extensively studied as that of other members of the ABC family. However, emerging evidence suggests that glycosylation plays crucial roles beyond its canonical role in the transport function. Specifically, glycosylation is implicated in preventing degradation and maintaining surface stability and dimerization. Furthermore, glycosylation influences the receptor affinity for ligands by modulating conformational changes within the protein structure [63].

3.2. CD26

Human CD26 primarily possesses nine potential N-glycosylation sites, whereas other species, such as rats, mice, bovines, and pigs, contain eight sites, with seven out of nine conserved among these species [64]. In rats, mutation of the sixth N-glycan impedes protein trafficking to the surface, leading to increased degradation alongside abolished enzymatic activity and preventing dimerization [65]. Conversely, another study found that none of the N-glycan modifications affect stability or enzymatic activity in human cell line systems [64]. This discrepancy may be related to the differences in species and cell systems used in the respective studies. Moreover, the fifth N-glycan position is crucial for ligand interaction [66]. To the best of our knowledge, aberrant glycosylation of CD26 in cancer remains unexplored; however, aberrant glycosylation at the eighth position is associated with Kashin–Beck disease, a degenerative osteoarthropathy [67]. Similarly to N-glycosylation, the O-glycan structure is important for CD26 stability. Lee et al. demonstrated that the deletion of

O-glycan synthetases reduced the surface expression of CD26, suggesting that O-glycan is important for CD26 localization at the plasma membrane [68]. The difference observed between studies in the biological function of glycosylation sites highlighted the need for further research to elucidate CD26 glycosylation in CSCs pathology.

3.3. CD66c

CD66c is heavily glycosylated, featuring numerous N-glycosylation sites that contribute to its structural and functional diversity. These glycans play crucial roles in mediating interactions with other proteins, cell adhesion, and signaling events. For instance, alterations in the glycosylation of CD66c have been observed to reduce heterodimer formation with CEACAM 8 [69]. Furthermore, distinct patterns of CD66c glycosylation have emerged as promising biomarkers in cancer progression. Specifically, the glycosylated form of CD66c has been correlated with a high recurrence rate in early stage oral squamous cell carcinoma patients. It has been shown to regulate signaling pathways associated with invasion and metastasis [70]. Sato et al. have developed a monoclonal antibody that selectively binds to the N-glycosylated form of CD66c on human colorectal spheroids and in patients. Notably, this antibody demonstrated an inhibitory effect on migration, while inducing cell detachment, suggesting that targeting glycosylated CD66c holds significant therapeutic potential [71].

Interestingly, O-glycan patterns on CD66s exhibit distinctions between normal and tumoral tissues. The application of the humanized antibody NEO-201, which selectively targets short O-glycan chains on CD66c, demonstrates robust binding to multiple cancer types while exhibiting negligible binding to adjacent healthy tissue [72,73]. Despite its importance, CD66c has been poorly explored compared to other members of the CEACAM family. Therefore, further research into the specific glycan structures and their functional consequences at distinct N-glycan and O-glycan sites on CEACAM6/CD66c will provide valuable insights into its diverse roles in CSCs.

3.4. uPAR

Human uPAR possesses five potential N-linked glycosylation sites, although only four have been observed to be glycosylated [74]. A mutation affecting all five N-linked glycosylation sites decreases the secretion levels of the soluble form. In contrast, deletion of the first N-linked glycan decreases receptor affinity for its ligand uPA [75]. Stahl et al. have demonstrated that the interaction between uPA and uPAR enhances cell migration of melanoma, while attachment of uPA–uPAR to the extracellular matrix, vitronectin, blocks migration and promotes melanoma adhesion [76]. These findings suggest that targeting the first N-glycan site could offer a promising strategy to counterbalance the pathogenic role of uPAR in promoting metastasis [48]. To the best of our knowledge, the O-glycan patterns were not explored on uPAR.

3.5. Trop-2

The transmembrane protein Trop-2 possesses four putative N-linked glycosylation sites (N-33, N-120, N-168, and N-208) located in the extracellular domain [77]. Direct mutagenesis experiments targeting different sites revealed that N-120 and N-208 play critical roles in ligand binding and exosomal release, while leaving surface expression unaffected [78]. While the aberrant glycosylation status of Trop-2 was not explored in cancer, aberrant glycosylation distribution was confirmed in transformed keratinocytes compared to normal cells [79]. Intriguingly, the subcellular distribution of Trop-2 serves as a determinant biomarker for cancer prognosis. The fully glycosylated form, characterized by predominant intracellular localization, correlates with a favorable outcome for patients with breast cancer, whereas surface expression is associated with an unfavorable prognosis [80,81]. To the best of our knowledge, the O-glycan patterns were not explored on Trop-2. These findings indicate that targeting N-linked glycosylation sites could serve as an appealing strategy to modulate its subcellular distribution and interactions with ligands.

Despite limited research into post-translational modifications for certain checkpoints, the glycosylation sites exposed at the surface represent promising targets for therapeutic intervention. Altogether, understanding how glycosylation impacts glycoprotein functionalities in CSCs can hold significant implications for the development of novel diagnostic tools and targeted therapies against CSCs.

4. Clinical Relevance and Translational Perspectives: Therapeutic Strategies

When addressing the resistance and metastatic capabilities of CSCs, selectively targeting surface metastatic checkpoints emerges as a promising strategy [10]. Developing specific targets against both entire proteins and their glycosylated forms holds the potential for selectively eradicating CSCs within the tumor bulk, thus preventing the recurrence of tumor growth and inhibiting invasion of distant sites. In this context, we provide an overview of the most promising in vivo and clinical therapies developed for each checkpoint (Table 2).

Table 2. Summary of therapeutic investigations.

Markers	Diseases	Therapy Name	Category	Systems/Clinical Trials	References
ABCB5	Melanoma	3C2-1D12	Monoclonal antibody	In vivo	[16]
CD26	Mesothelial cancer, renal carcinoma, urothelial cancer	YS110	Monoclonal antibody	Phase I NCT03177668	[82]
	Diabetes with advanced colorectal and airway cancers	sitagliptin	Small molecule inhibitor	observational studies	[83]
CD66c	Pancreatic cancer	CAR-T cells	Cell-based therapy	In vivo	[84]
	Non-small cell lung cancer	2Ab & 4Ab	Multivalent antibodies	In vivo	[43]
uPAR	Pancreatic, colorectal prostate, ovarian, breast cancers	ATN-658	Humanized antibody	In vivo	[85–89]
	Pancreatic cancer	ATN-292	Humanized antibody	In vivo	[85]
	Non-small cell lung cancer	CAR-T cells	Cell-based therapy	In vivo	[90]
	Breast and colon cancer	MV-h-uPA	Oncolytic virotherapy	In vivo	[91–93]
Trop-2	Triple-negative breast cancer	Sacituzumab Govitecan	Antibody–drug conjugate	FDA approved, NCT03901339	[94]
	Epithelial cancers	Sacituzumab Govitecan	Antibody–drug conjugate	Phase I and II NCT01631552	[95]
	Triple-negative breast cancer	Dato-DXd	Antibody–drug conjugate	Phase III NCT05104866	[94]
	Non-small cell lung cancer	Dato-DXd	Antibody–drug conjugate	Phase III NCT05687266	[96]
	Gastric cancer	CAR-T cells	Cell-based therapy	In vivo	[97]
	Solid cancer	CAR-T cells	Cell-based therapy	In vivo	[98]

In a melanoma xenograft model, treatment with an anti-ABCB5 monoclonal antibody resulted in a notable reduction in tumor formation and growth, accompanied by prolonged protection against tumor initiation. Mechanistically, the anti-ABCB5 antibody was found to promote antibody-dependent cell-mediated cytotoxicity (ADCC), specifically in ABCB5-positive CSCs, underscoring its immune effector properties. However, despite its inhibitory effect, residual ABCB5-positive cells persisted in a few cases (27%), implying limited accessibility of the antibody to the ABCB5 target [16].

A phase I clinical trial investigating YS110, a high-affinity antibody targeting CD26, in patients with advanced or refractory solid tumors (e.g., 22 patients with mesothelial cancers, 10 renal cell carcinoma, and one urothelial carcinoma, NCT03177668) has yielded promising results, demonstrating significant anti-tumor effects alongside favorable safety profiles [82]. Similar clinical outcomes were found in another phase I clinical trial conducted in Japan

on a lower cohort (e.g., nine patients with malignant pleural mesothelioma) [99]. Notably, the soluble form of CD26 shows potential as a biomarker for monitoring YS110 therapy efficacy, as its expression decreases following YS110 administration, indicating treatment response. It is worth mentioning that YS110 does not bind to the catalytic site of CD26, thereby leaving its enzymatic activity unaffected [100]. Furthermore, sitagliptin, a CD26 inhibitor, exhibited anti-tumor activity in patients with concurrent diabetes and advanced colorectal or lung cancer [83].

Schäfer et al. developed chimeric antigen receptors T cells (CAR-T) targeting CD66c and assessed their efficacy both in vitro and in vivo in pancreatic cancer. The CD66c-CAR-T cells demonstrated robust functional activation and cytotoxicity in vitro. Interestingly, the study investigated various spacer lengths for CAR-T constructs, and found that shorter spacers exhibited superior therapeutic outcomes in vivo compared to longer spacers. The observed low responsiveness was attributed to limited target availability and inadequate infiltration of CAR-T cells at the tumor site [84]. In addition, previous studies indicated that longer spacers are more effective for targeting molecules close to the membrane and, conversely, shorter spacers are advantageous for other epitopes [101]. These findings highlight the significance of considering both the localization and expression level of the target when designing CAR-T cells. In a separate study, the utilization of multivalent antibodies against CD66c showed significant inhibition of invasion and metastasis in vitro, along with reduced tumor growth in a lung cancer xenograft model [43].

Numerous therapeutic strategies have emerged targeting uPAR, including monoclonal antibodies, CAR-T cell-based therapy, and oncolytic virotherapy [102]. Here, we present notable examples of effective therapeutic approaches. For instance, ATN-658, a humanized antibody, selectively targets uPAR without disrupting its binding to the uPA ligand. Multiple studies have demonstrated the promising anti-tumor effects of ATN-658 across various in vivo tumor models. It effectively suppresses tumor growth and metastasis in pancreatic [85], colorectal [86], prostate [87], ovarian [88] and breast cancer [89]. Furthermore, its efficacy is enhanced when combined with drug therapy [85,88,89]. Similarly, ATN-292, which disrupts the interaction between uPAR and uPA, reduces the metastatic properties of pancreatic cancer [85]. In the realm of immunotherapy, Amor et al. engineered anti-uPAR CAR-T cells aimed at targeting senescent cells expressing uPAR in lung cancer. Their study demonstrated that CAR-T cells effectively diminished uPAR-positive tumor cells, prolonged the survival rate of mice, and increased immune cell infiltration [90]. Additionally, the advancement of oncolytic virotherapy presents a promising approach to cancer treatment by utilizing viruses to infect and eradicate cancer cells while preserving normal tissue selectively. In this context, the measles virus (MV) has been engineered to target uPAR (MV-h-uPA) in various in vivo tumor models. MV-h-uPA significantly suppressed tumor growth in breast cancer [91], and extended survival in the murine colon and mammary cancers [92], while also reducing metastasis to distant tissues [93].

Targeting Trop-2 has emerged as a promising avenue for the treatment of refractory cancers. Sacituzumab Govitecan (IMMU-132) stands out as a novel anticancer medication that effectively combines an antibody with a potent cytotoxic agent. Classified as an antibody–drug conjugate (ADC), its components include the humanized antibody hRS7, designed to target the Trop-2 antigen, and a derivative of the topoisomerase chemotherapy inhibitor SN-38, known as irinotecan. This unique combination allows Sacituzumab to selectively target and eliminate cancer cells expressing Trop-2 while minimizing damage to healthy tissues. By precisely targeting cancer cells, this approach holds the potential to reduce the adverse effects commonly associated with traditional chemotherapy. In 2019, IMMU-132 received approval from the United States Food and Drug Administration (FDA) for triple-negative breast cancer (TNBC) patients who have been refractory to two or more prior therapies (NCT03901339) [94]. Ongoing clinical trials for Sacituzumab Govitecan primarily focus on treating patients with metastatic forms in breast, brain, epithelial, lung, prostate, and urothelial cancers, as well as other malignancies (NCT01631552, [95]). As another ADC targeting Trop-2 and associated with topoisomerase inhibitor (DXd),

Datopotamab deruxtecan (Dato-DXd) is another Trop-2-directed ADC with potent DNA topoisomerase I inhibitor (DXd), and is currently tested in a phase III clinical trial in triple-negative breast cancer (NCT05104866, [94]) and NSCLC (NCT05687266, [96]). In addition to antibody-targeted therapies, two CAR-T cell therapies targeting Trop-2 have demonstrated promising results in vivo. Zhao et al. developed a bi-specific Trop-2 and PD-L1 CAR-T cell, which effectively reduced tumor growth in gastric xenograft models compared to single Trop-2-specific CAR-T cells [97]. Similarly, Chen et al. found that the anti-tumor activity of Trop-2-specific CAR-T cells is enhanced when associated with the co-stimulatory molecule CD27 [98]. These findings suggest that optimizing the therapeutic efficacy of Trop-2-specific CAR-T cells relies heavily on engineered receptors and co-factors. A range of advanced therapies targeting Trop-2 is presently undergoing in vitro and in vivo validation, including monoclonal antibodies, bispecific antibodies, antigen-conjugated nanoparticles, and virus-like particles [54,55].

Overall, numerous therapeutic strategies have been devised against these markers, yielding promising outcomes. Nevertheless, some questions persist regarding the effectiveness of these therapies in eradicating residual cells and preventing tumor re-initiation.

5. Targeting Glycosylation for Personalized Therapy in CSCs

Targeting aberrant glycosylation in cancer cells presents a multifaceted therapeutic approach. Various strategies have been developed to disrupt different stages of the glycosylation processes, with the aim of reducing their binding activity and functionality or by reducing the synthesis of aberrant glycan structures [103].

Since many therapies, cited above, target the entire antigen using blocking antibodies, there is a need to explore targeting specific glycans within glycoproteins inspired by approaches developed for PD-1 and PD-L1 glycosylated patterns, which could enhance specificity towards target cells and narrow the therapeutic focus to only CSCs. For example, Sun et al. demonstrated the N-glycan at position 58 in PD-1 is responsible for maintaining its stability and facilitating interaction with PD-L1. By employing the glycosylation-targeting blocking antibody STM418, they observed a marked enhancement in anti-tumor activity, both in vitro and in vivo, attributed to the disruption of PD-1/PD-L1 pairing. Another strategy, inspired from targeting glycosylated PD-L1, involves disrupting the modulator of glycosylation processes such as the N-glycosyltransferase STT3. Targeting STT3 with anti-cancer agents disrupts PD-L1 stability on the CSCs surface, effectively suppressing immune evasion by CSCs [104–106].

Additionally, recent technologies using gene editing techniques can also offer further therapeutic avenues. The CRISPR-Cas9 genome editing technologies can accurately target and edit genes in cancer, modulating cancer cell behavior, including proliferation, metastasis, and resistance to therapy [107]. While CRISPR-Cas9 studies on cancer focus on in vitro and in vivo models, the genome editing provides a powerful tool for identifying genes implicated in metastasis and drug resistance. Through systematic gene perturbation in cancer cells, CRISPR-Cas9 screens can unveil novel drug targets and mechanisms of resistance, thereby informing the development of more effective cancer therapies by assessing their impact on drug sensitivity [108]. Hence, by editing these surface markers or enzymes involved in glycosylation mechanisms, precision genome editing can modulate the expression or function of these cancer stem cell surface markers, potentially leading to more personalized strategies against CSCs [109].

With the advancement of targeted therapies for cancer, new avenues may open up to specifically target cancer-associated surface proteins or aberrant glycosylation signatures, minimizing off-target effects and improving therapeutic efficacy [110].

6. Conclusions

In the present review, we have presented current knowledge about the glycoproteins ABCB5, CD26, CD66c, uPAR, and Trop-2, which emerge as intriguing surface markers to investigate in tumorigenesis. These glycoproteins stand as critical metastatic checkpoints

and contributors to drug resistance in the biology of CSCs. Despite the initiation of promising clinical trials for several targets, the exploration of glycan patterns is still in the early phase. Comparative therapy, evaluating efficacy between CSCs and non-CSCs, is essential for progressing towards personalized targeting of cancer subtypes, tissues, and individual patients. Continued exploration of the intricate network of glycosylation governing CSC biology holds immense promise for advancing personalized medicine, ultimately leading to enhanced outcomes for cancer patients.

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