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Tubulin Isotypes: Emerging Roles in Defining Cancer Stem Cell Niche

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Although the role of microtubule dynamics in cancer progression is well-established, the roles of tubulin isotypes, their cargos and their specific function in the induction and sustenance of cancer stem cells (CSCs) were poorly explored. But emerging reports urge to focus on the transport function of tubulin isotypes in defining orchestrated expression of functionally critical molecules in establishing a stem cell niche, which is the key for CSC regulation. In this review, we summarize the role of specific tubulin isotypes in the transport of functional molecules that regulate metabolic reprogramming, which leads to the induction of CSCs and immune evasion. Recently, the surface expression of GLUT1 and GRP78 as well as voltage-dependent anion channel (VDAC) permeability, regulated by specific isotypes of β-tubulins have been shown to impart CSC properties to cancer cells, by implementing a metabolic reprogramming. Moreover, βIVb tubulin is shown to be critical in modulating EphrinB1signaling to sustain CSCs in oral carcinoma. These tubulin-interacting molecules, Ephrins, GLUT1 and GRP78, are also important regulators of immune evasion, by evoking PD-L1 mediated T-cell suppression. Thus, the recent advances in the field implicate that tubulins play a role in the controlled transport of molecules involved in CSC niche. The indication of tubulin isotypes in the regulation of CSCs offers a strategy to specifically target those tubulin isotypes to eliminate CSCs, rather than the general inhibition of microtubules, which usually leads to therapy resistance.

Keywords: tubulin, tubulin-interacting proteins, cancer stem cell niche, metabolic reprogramming, immune evasion, GLUT1, GRP78, EphrinB1

INTRODUCTION

Microtubules, a major class of the cytoskeleton of cells, are formed of heterodimers of α and β tubulins (1). In addition to the heterogeneity of the tubulin isotypes forming the dimers, their post-translational modifications and interacting proteins influence the dynamics of microtubules (2, 3). As microtubules regulate plethora of cellular processes, their deregulation is associated with diseases, including cancer. As the field evolves, it appears that the tubulin isotypes might have unique functions specified by their interacting molecules, which again is defined by their unique C-terminal end sequence and the post translational modifications (PTMs) therein (2). Deregulation of some of the tubulin isotypes in cancer suggests their involvement in cancer progression. Though microtubule targeting agents (MTA) have been used in cancer treatment for a long time, the role of

the tubulin isotypes in the cancer stem cell (CSC) context was not explored extensively. Some of the recent findings suggest that specific tubulin isotypes have some important roles in regulating certain functionally essential molecules involved in the induction and maintenance of CSCs (4–8). This review focuses on the molecules that are shown to interact with tubulin isotypes, probably their cargos. We discuss the recent reports portraying the role of tubulin isotypes and their plausible cargos in metabolic reprogramming, induction of CSCs and modulation of immune evasion

THE TUBULIN CODE

Several isotypes of tubulin are found for both α- and β-tubulins in different species. So far, mammals are known to have nine α-tubulin and nine β-tubulin genes, (**Figure 1**) (for nomenclature see: www.genenames.org/cgi-bin/genefamilies/set/778). The isotype composition, differing majorly in the 20 amino acids of their extremely acidic C-terminals, plays a critical role in imparting structural conformation, motor activity and tunes the microtubule dynamics (1, 3), which turns out to be the reason for the unique functions of particular tubulin isotype combinations. The concept that molecular patterns generated by combinations of tubulin isotypes and PTMs is termed the 'tubulin code'. The tubulin molecule with highly conserved core can have slightly different structures with different isotype incorporation affecting microtubule assembly, dynamics and mechanical properties. Also, the isotypes with unique flexible tails that protrude outward from the surface of microtubules affect the interaction with microtubule associating proteins (MAPs) and thus can cause unique PTMs. Further, this variation is multiplied by a plethora of PTMs such as acetylation, tyrosination, detyrosination, glutamylation, polyglutamylation, glycation, phosphorylation etc (2). Isotype overexpression or down-regulation can bring in several diseases including neuronal disorders (9), cancer (10), drug resistance (11, 12) etc. So far it was not possible to assign the sequence difference of specific isotypes to different functions but it can be assessed that the variable stretch of negative charges over the C-terminus along with PTMs incorporated might play a significant role in defining the unique functions.
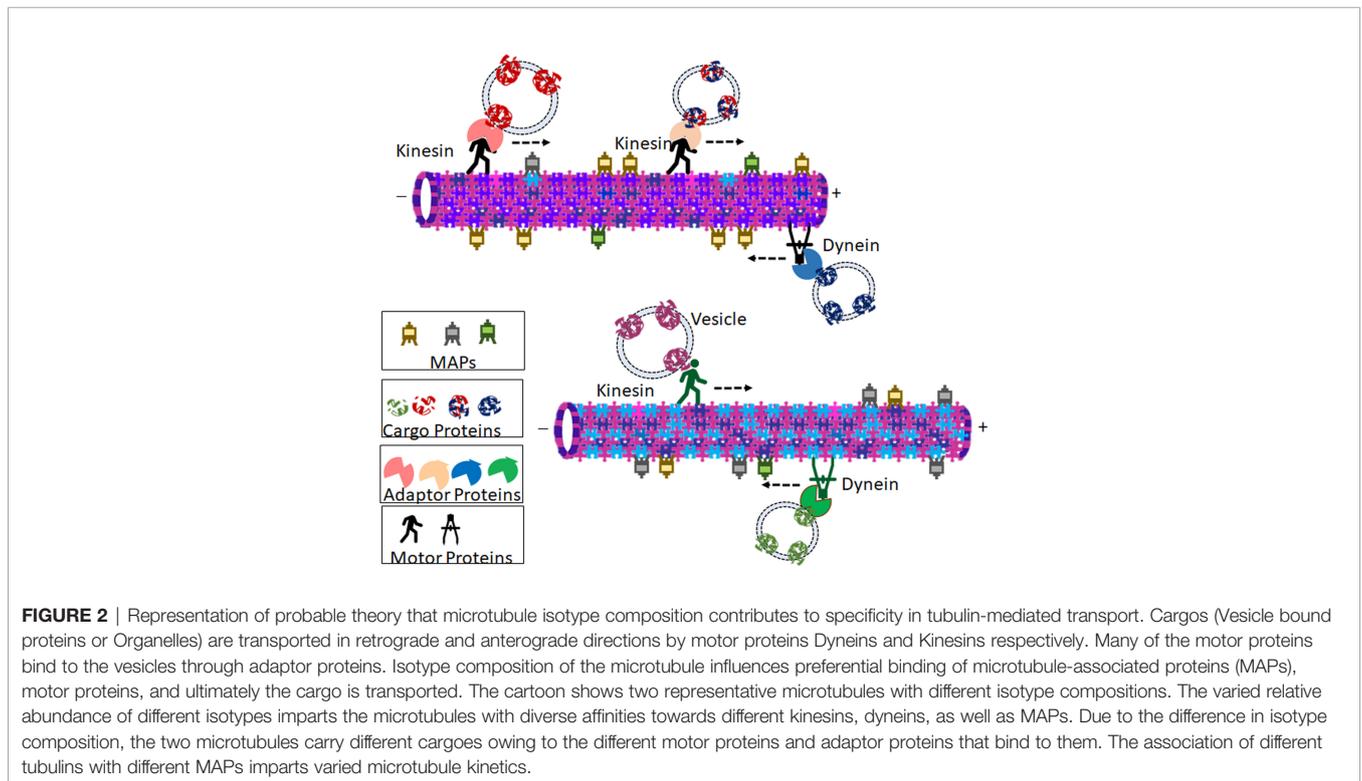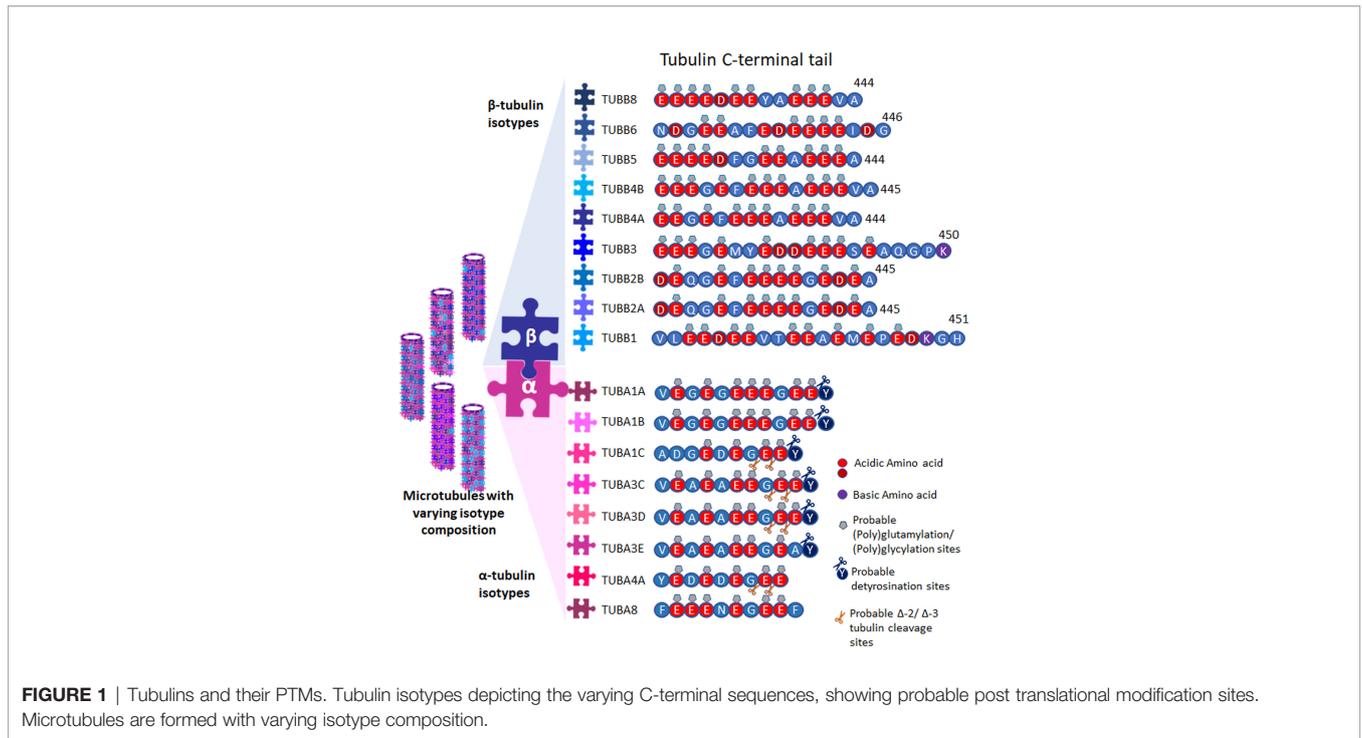
MICROTUBULE-MEDIATED TRANSPORT

Microtubules act as tracks for long-range intracellular transport of many important vesicles and organelles through its motor proteins kinesins and dyneins (**Figure 2**). They are critical in the positioning of golgi complex (13), endoplasmic reticulum motility (14), long-distance transport of mitochondria (15) as well as the translocation and clustering of endosomes and lysosomes (16, 17). Microtubules mediated trafficking has negative effects also. For example, pathogenic viral cargos require microtubules to transport them to and from their intracellular replication sites. The microtubule-mediated transport of cargo with spatiotemporal specificity and efficiency demands the involvement of a set of other proteins. Some

of the MAPs, identified initially as the proteins that bind to and stabilize microtubules, specifically Tau, MAP1B, MAP2, MAP4, MAP6, and MAP7, are now considered to be the regulators of intracellular traffic (18). The MAPs, specifically the plus-end-tracking proteins (+TIPs) that respond to various cellular signals, regulate the dynamic behavior and organization of the microtubule tracks (19). While the motor proteins power the transport, modifications of adapter proteins-the molecules that recruit cargos to the motor- fine tunes the specificity of transport (20). At the same time, some cargos can directly interact with their motors (21). Septins are multimeric GTPases that function as adapter proteins, which brings about the selective recruitment of microtubule motors to their respective cargo (22). Another such adapter molecule is c-jun NH_2-terminal kinase (JNK)–interacting proteins (JIPs), which are scaffolding proteins for the JNK signaling pathway (23). JIP1, the kinesin-1 cargo, is localized only to a subset of neurites in cultured neuronal cells. This polarized protein trafficking appears to involve the preferential recognition of kinesin-1 motor domain to microtubules containing specific posttranslational modifications (PTMs) such as α-tubulin acetylation at Lys-40 (24). However, this PTM alone is not sufficient to affect kinesin-1 velocity and run length (25). It is becoming increasingly evident that the tubulin code with different isotype composition and posttranslational modifications plays an extremely important role in controlling motor behaviors and the dynamicity of microtubules (1, 26). Yet, despite decades of extensive research, our knowledge on the spatiotemporal regulation of microtubule is incomplete (27). The mechanism of cargo specificity during microtubule transport is still a mystery, as there are fewer known adapters than the number of cargos (27). In this context, some of the recent reports of the direct interaction of some functional proteins, like VDAC, N-Cadherin, GLUT1 and EphrinB1, to specific tubulin isotypes, suggests additional mechanism of implementing specificity of transport (4–6, 28).

TUBULINS IN CANCER

Consistent with the divergent role of tubulins in cell cycle regulation, apoptosis and drug resistance, their deregulation has been reported in a wide range of cancers. Aberrant expression of certain isotypes of tubulins in cancer tissue specimens are reported to regulate cancer progression, metastasis, aggressive behavior, drug resistance or poor prognosis, as summarized in **Table 1**. The upstream signaling leading to this aberrant expression is not well-characterized except for βIII-tubulin (44). As summarized in a recent review, several factors including hormones, bromodomain and extraterminal (BET) proteins, factors like hypoxia and hypoglycemia can up-regulate the expression of βIII-tubulin (44). While several upstream signaling pathways like AKT, K-RAS and EGFR upregulate its expression, tumor suppressor PTEN negatively regulates the gene expression (44). The upstream pathways that regulate the PTMs of the rest of the tubulin isotypes are yet to be unraveled.

The role of different isotypes of tubulins in cancer progression is reviewed recently (45, 46). It is well-established that

FIGURE 1 | Tubulins and their PTMs. Tubulin isotypes depicting the varying C-terminal sequences, showing probable post translational modification sites. Microtubules are formed with varying isotype composition.

FIGURE 2 | Representation of probable theory that microtubule isotype composition contributes to specificity in tubulin-mediated transport. Cargos (Vesicle bound proteins or Organelles) are transported in retrograde and anterograde directions by motor proteins Dyneins and Kinesins respectively. Many of the motor proteins bind to the vesicles through adaptor proteins. Isotype composition of the microtubule influences preferential binding of microtubule-associated proteins (MAPs), motor proteins, and ultimately the cargo is transported. The cartoon shows two representative microtubules with different isotype compositions. The varied relative abundance of different isotypes imparts the microtubules with diverse affinities towards different kinesins, dyneins, as well as MAPs. Due to the difference in isotype composition, the two microtubules carry different cargoes owing to the different motor proteins and adaptor proteins that bind to them. The association of different tubulins with different MAPs imparts varied microtubule kinetics.

microtubule network and their dynamics are important regulators of mitosis and cell proliferation (45). Since microtubule dynamics also plays a role in cell migration, tubulins, more specifically their post-translational modifications, regulate invasion and metastasis (34, 45, 47). Further, tubulins also play a critical role in the regulation of drug resistance (45). The most studied post-translational modification of tubulins that regulate different cancer

TABLE 1 | Tubulin deregulation in cancer.

Isotype	Alteration	Cancer	Outcome	Reference
α-tubulin*	Loss of expression	Breast cancer	Metastasis	(29)
	Increased acetylation	Breast cancer	Metastasis, Aggressive behavior	(30)
αIa-tubulin, αIb-tubulin	Over-expression	Breast cancer	Taxane resistance	(31)
βI-tubulin	Over-expression	Breast cancer	Docetaxel resistance	(32)
	Over-expression	Breast cancer	Taxane resistance	(31)
βII-tubulin*	Increased mRNA	Nasopharyngeal carcinoma	Cancer progression	(33)
	Over-expression	Colorectal cancer	Poor outcome	(34)
	Nuclear localization	Variety of cancers	Metastasis	(35)
βIIa-tubulin	Expression	Breast cancer	Metastasis	(36)
βIIb-tubulin	mRNA expression	Colorectal cancer	Poor survival	(37)
	mRNA expression	Renal cancer	Poor survival	(38)
	Down-regulation	Breast cancer	Taxane resistance	(31)
βIII-tubulin	Over-expression	Breast cancer	Docetaxel resistance	(32)
	Over-expression	Breast cancer	Taxane resistance	(31)
	Over-expression	Clear cell renal carcinoma	Poor prognosis	(39)
	Over-expression	Prostate cancer	Docetaxel resistance	(40)
	Over-expression	Colorectal cancer	Poor prognosis	(41)
	Over-expression	HNSCC	Unrelated to clinical outcome	(42)
βIVb-tubulin	High mRNA Expression	HNSCC	Unrelated to clinical outcome	(5)
βV-tubulin	Over-expression	NSCLC	Prolonged progression-free survival	(43)
	Down-regulation	Breast cancer	Taxane resistance	(31)

*Reported deregulation of Tubulins in tissue samples of different cancers. Data from cell lines are not included. HNSCC, Head and neck squamous cell carcinoma; NSCLC, non-small cell lung carcinoma. *References where isotypes are not mentioned.*

properties is acetylation. Acetylation of different isotypes of tubulin is shown to regulate the invasive property, metastatic ability and resistance to chemotherapy (30, 48). The role of different posttranslational modifications, including acetylation, detyrosination, tyrosination, polyglutamylation, and polyglycylation in the regulation of cancer properties are extensively reviewed elsewhere (49).

The classical research on the role of tubulins in cancer revolves around the microtubule dynamics that change the biophysical properties of the cancer cell. But recent evidences implicate that tubulins can indirectly regulate many cancer properties including drug resistance, metastasis and immune evasion by the transport of important molecules necessary for the maintenance of cancer stem cells (CSCs) and their niche (4–8). Majority of the studies in the field were focused on the tubulin-interacting molecules that regulate the dynamics of microtubules like motor proteins and MAPs (50). Yet, there are some studies that showed that molecules other than microtubule associated proteins and motor proteins co-immunoprecipitates with specific tubulins, suggesting the specificity of isotypes for selecting the cargo (**Table 2**). So far, there is only one study reported using proteomic approach to identify the interacting partners of βIII-tubulin, which revealed that the molecule forms complexes with important regulators like GRP78, Vimentin and GSTM4 in cancer cells (56). Interestingly, nuclear βII-tubulin is shown to associate with Notch1 intracellular domain (55). Recently, it is also shown that S100A6 binds to alpha and beta tubulins, and the secretion of S100A6 is dependent on its tubulin-binding (7). Another important observation linking tubulins to mitochondrial bioenergetics function is the regulation of voltage-dependent anion channel (VDAC) permeability by βII-tubulin and βIII-tubulin (4, 57). Also, it was found that βIVb-tubulin interacts with GLUT1 in the CSC

context (6). Moreover, we recently reported the involvement of βIVb-tubulin in the possible transport of EphrinB1 in the CSC niche (5). **Table 2** gives a summary of the known interacting molecules of tubulins, which might have a role in the regulation of cancer properties. Though the role of respective tubulins in their transport is not well-established in certain cases, it opens up the possibility of tubulins playing a role in specific transport of important intermediates of proliferation, apoptosis, chemoresistance, CSC properties and immune evasion. In the following sections, we will elaborate on how the transport function of tubulins can possibly regulate the cancer properties.

TUBULINS AND THEIR INTERACTING PROTEINS IN THE REGULATION OF CANCER PROGRESSION

Given the role of tubulins in the regulation of mitosis, a number of chemically diverse substances are developed that bind to tubulin and inhibit cell proliferation by disrupting the microtubule dynamics, activating spindle assembly checkpoints and mitotic arrest (58). The recent advances in the field suggest that tubulin isotypes other than γ-tubulins might have a microtubule-mediated spindle assembly-independent role in proliferation (54). Although the voltage-gated potassium channel EAG2 is shown to interact with α-tubulin and β-tubulin, the possible role of this interaction in its trafficking and function in tumor progression is yet to be studied (54). However, EAG2 plays a role in cancer progression, as shown in medulloblastoma (59). It is shown to be up-regulated in medulloblastoma tissues and its knock-down impairs medulloblastoma cell growth *in vitro*, reduces tumor burden *in*

TABLE 2 | Tubulin interacting proteins.

Tubulin type	Interacting Molecule	Interaction context	Reference	Relevance of interacting molecule in cancer
α-tubulin	Vimentin	Colon cancer migration	(51)	Metastasis
	VDAC1	Lung cancer cells	(52)	Metabolic reprogramming in cancer
α-tubulin and βI-tubulin	RAMP1	Mouse TSA cells and Human SH-SY5Y neuroblastoma cells	(53)	Increase proliferation
α-tubulin and β-tubulin	EAG2	Human brain medley	(54)	Increase proliferation and cancer progression
α-tubulin and β-tubulin	S100A6	Regulates secretion of S100A6 in mesenchymal stem cells, WJMS	(7)	Metastasis
αIa-tubulin, and βIVb-tubulin	Connexin43	Mouse brain	(8)	Increase proliferation, metastasis, inhibit apoptosis
β-tubulin	Connexin43	HeLa cells	(8)	Increase proliferation, metastasis, inhibit apoptosis
βII-tubulin	Notch1-NIC	Nuclear translocation of Notch in Leukemia cells	(55)	Regulate CSCs
βIII-tubulin	Vimentin	Ovarian cancer cells	(56)	Metastasis
	GRP78	Ovarian cancer cells	(56)	Regulate CSCs
	GRP75	Ovarian cancer cells	(56)	Regulate proliferation, survival and CSC properties
	GSTM4	Ovarian cancer cells	(56)	Drug resistance
βIII-tubulin and βIVa-tubulin	N-Cadherin	Endothelial cells	(28)	Metastasis
βIVb-tubulin	GLUT1	Glioblastoma stem cell niche	(6)	Regulate CSCs
	EphrinB1	Oral cancer stem cell niche	(5)	Regulate CSCs

The table enlists molecules that are reported to interact with tubulins, confirmed by immunoprecipitation.

vivo and enhances survival in xenograft studies (59). Connexin43 is a gap junction protein shown to be associated with tubulins, and it is involved in cancer progression by modulating MAPK and JNK pathways (60–63). One of the other properties attributed to tubulins is cell migration. Hence, it is implicated in metastasis and tubulin inhibitors are shown to block metastasis in various cancers (47, 64, 65). Now there are accumulating evidences to show that tubulins can regulate cancer properties other than proliferation and metastasis. Some of the recent studies show that certain isotypes of tubulins are associated with CSCs, and the knock-down of those isotypes can deplete CSCs (5, 6, 39).
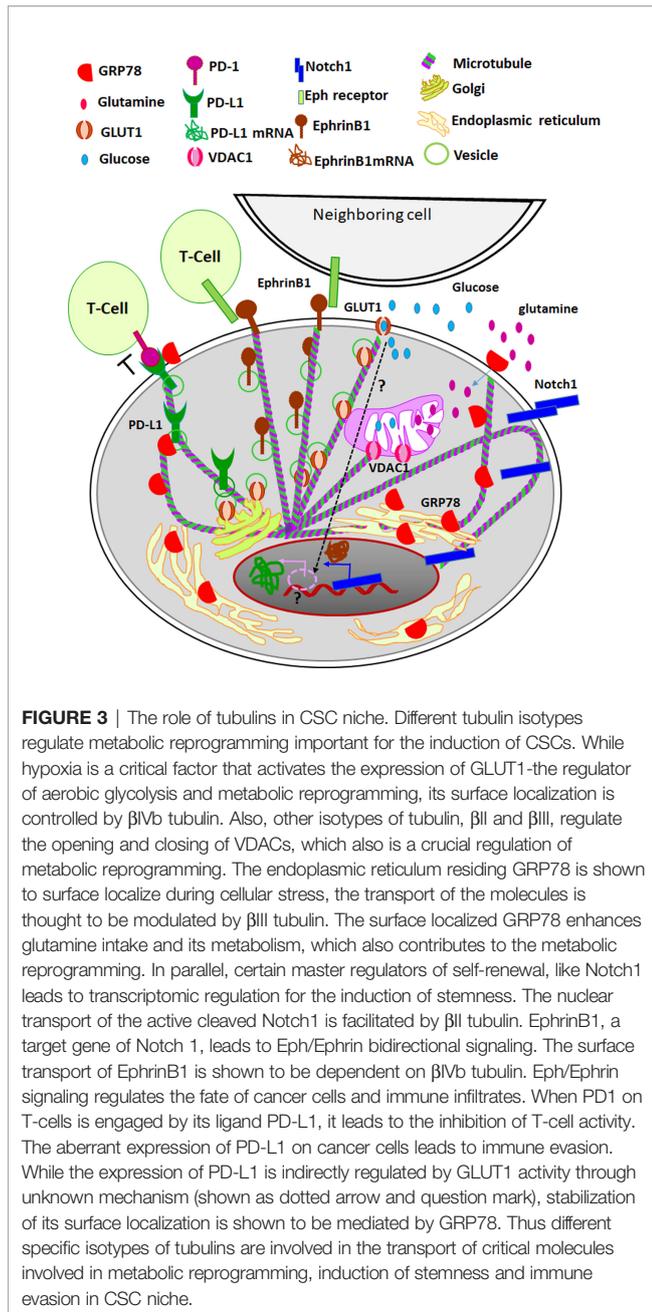
TUBULINS IMPLICATED IN THE REGULATION OF CSCS AND THEIR NICHE

As summarized in **Table 1**, specific isotypes of tubulins are enriched in certain cancers, which lead to the acquisition of resistance to tubulin-binding agents. Thus, several studies have investigated the role of different isotypes in imparting resistance, which revealed that the dynamics of βIII tubulin comprising microtubule are quite different from the ones composed of mixed β tubulins (10, 46). Further, binding efficiencies of different microtubule-targeting agents (MTA) to microtubules comprising βIII tubulins are lower compared to their efficiencies to bind to microtubules consisting of different β tubulins (10, 46). Though the expression profiles of tubulin isotypes in cancer and chemoresistance are well-explored, the significance of tubulin isotypes in the regulation of CSCs is poorly studied. However, a recent study using several glioblastoma cell lines has shown that there was a reduction in the detyrosinated form of α-tubulin,

acetylated α-tubulin and phosphorylated βIII-tubulin with a concomitant up-regulation of polyglutamylated α and β-tubulins in MTA-resistant cells compared to sensitive cells. Also, the MTA-tolerant cells expressed stemness markers, suggesting that CSCs exhibit resistance to MTA (66). The MTA-resistant glioblastoma cell lines had a relative enrichment of βII and βIII tubulins, while the detyrosination and the change in Δ-2 α-tubulin levels were not correlated to resistance (66). Shortly after that, a more direct evidence of the role of βIII tubulins in clear cell renal cell carcinoma stem cells was reported (39). They showed that there is a positive correlation between the expression of βIII tubulin and stem cell markers (39). In accordance with that, the depletion of βIII tubulin resulted in the loss of CSC properties (39). Recently, our studies in oral cancer have shown that βIVb-tubulin is indispensable for the maintenance of CSCs, specifically in defining the CSC niche (5). Further, it was found to be critical for the maintenance of glioblastoma stem cells (6). A detailed analysis of the recent literature suggest that different tubulin isotypes are involved in the regulation of CSCs and their niche, possibly by the transport of essential molecules that regulate CSC properties. The important CSC-regulating molecules that are suggested to be transported by tubulins are signaling molecules like, Ephrins and Notch; regulators of metabolism like, GLUT1, GRP78 and VDAC (**Table 2**). Many of these molecules present in CSC niche are important in establishing metabolic reprogramming, imparting self-renewal ability and to some extent, to facilitate immune evasion (**Figure 3**).

Metabolic Reprogramming in CSC Niche

In order to meet the requirements of exponential growth and proliferation, cancer cells adapt to aerobic glycolysis, glutamine catabolism, *de novo* lipid synthesis and nucleotide synthesis, which is generally known as metabolic reprogramming (67). The switch of tumor cellular bioenergetics from an oxidative

FIGURE 3 | The role of tubulins in CSC niche. Different tubulin isotypes regulate metabolic reprogramming important for the induction of CSCs. While hypoxia is a critical factor that activates the expression of GLUT1-the regulator of aerobic glycolysis and metabolic reprogramming, its surface localization is controlled by βIVb tubulin. Also, other isotypes of tubulin, βII and βIII, regulate the opening and closing of VDACs, which also is a crucial regulation of metabolic reprogramming. The endoplasmic reticulum residing GRP78 is shown to surface localize during cellular stress, the transport of the molecules is thought to be modulated by βIII tubulin. The surface localized GRP78 enhances glutamine intake and its metabolism, which also contributes to the metabolic reprogramming. In parallel, certain master regulators of self-renewal, like Notch1 leads to transcriptomic regulation for the induction of stemness. The nuclear transport of the active cleaved Notch1 is facilitated by βII tubulin. EphrinB1, a target gene of Notch 1, leads to Eph/Ephrin bidirectional signaling. The surface transport of EphrinB1 is shown to be dependent on βIVb tubulin. Eph/Ephrin signaling regulates the fate of cancer cells and immune infiltrates. When PD1 on T-cells is engaged by its ligand PD-L1, it leads to the inhibition of T-cell activity. The aberrant expression of PD-L1 on cancer cells leads to immune evasion. While the expression of PD-L1 is indirectly regulated by GLUT1 activity through unknown mechanism (shown as dotted arrow and question mark), stabilization of its surface localization is shown to be mediated by GRP78. Thus different specific isotypes of tubulins are involved in the transport of critical molecules involved in metabolic reprogramming, induction of stemness and immune evasion in CSC niche.

phosphorylation to aerobic glycolytic pathway is now recognized as one of the hallmarks of cancer (68). At the same time, it is also shown that CSCs have metabolic plasticity, and can switch between mitochondrial respiration and glycolysis (69) Research on this topic for the last two decades has shown that metabolic reprogramming is the key to the induction of CSCs (70, 71). The most convincing link between tubulins, cellular bioenergetics and CSCs is the role of tubulin-VDAC axis in the metabolic reprogramming in cancer cells (72). VDACs are located in the mitochondrial outer membrane, which function as a metabolic link between glycolysis and oxidative phosphorylation (72). Both

βII and βIII tubulins are found to regulate VDAC channel permeability in normal and cancer cells (57). The dynamic regulation of free and dimerized tubulins regulate VDAC opening and closing to modulate mitochondrial metabolism, reactive oxygen species formation, and the intracellular flow of energy (72). In accordance with that, cytoskeleton-mitochondrial interactions through VDACs are implicated in the regulation of CSCs (73).

One of the first reports that link microtubules to glucose metabolism is the observation that D-glucose induces tyrosination of tubulins (74). The tyrosination of tubulins, in turn, has been shown to regulate the motor protein-mediated transport in the nervous system (75, 76). Following up the research on the transport function of tubulins unraveled the role of tubulins in the transport of many molecules involved in the regulation of glucose metabolism in CSCs and their niche. Hypoxia, the most important factor leading to the metabolic reprogramming and induction of CSCs, is shown to induce GLUT1, the transporter of glucose into cells (77, 78). The metabolic reprogramming in CSCs is shown to be dependent on GLUT1 in many cancer types (6, 79–81). Remarkably, GLUT1 is shown to interact with βIVb-tubulin in glioblastoma specimens using mass spectrometric analysis, which was confirmed again by proximity ligation assay and immunoprecipitation (6). Of note, this depletion of the tubulin reduced the surface expression of GLUT1, and resulted in the loss of CSC properties (6), thereby highlighting the role of βIVb-tubulin in the membrane transport of GLUT1 and the regulation of stem cells thereby. Like GLUT1, another important regulator of metabolic reprogramming, GRP78, is shown to associate with tubulins. It is shown that βIII tubulin interacts with GRP78, and this interaction is critical for the survival of cancer cells in the glucose starved condition by adapting to use other nutrient supplies present in the tumor microenvironment (82). This GRP78-mediated survival is shown to be dependent on the enhanced glutamine catabolism (83). Thus, GRP78, more specifically the cell surface GRP78, regulates metabolic reprogramming, which depends on several other molecular players (84, 85). Whether the reported interaction of tubulins to GRP78 plays any role in its cell surface function is yet to be studied.

Regulation of Self-Renewal of CSCs

The over-expression of βII tubulin and its nuclear localization is reported as a marker for poor prognosis in colorectal cancer (34). The importance of this βII tubulin-nuclear localization in the aggressive nature of the malignancy was explained by another study that demonstrated the involvement of tubulin βII in the nuclear transport of Notch1 and its CBF-dependent transcriptional activity (55). Notch1 is supposed to be a master-regulator of stem cell properties, as it controls a variety of molecules that regulate CSCs and their niche in various malignancies (86–89). Notch signaling is important in the maintenance of stem cells in intestinal crypts, by regulating the expression of EphrinB1, where the reciprocal gradients of EphB2 and EphrinB1 define the balance of intestinal stem cell self-renewal and differentiation (90). In cancer context also Notch1 is reported to regulate EphrinB1 signaling, as shown in osteosarcoma (91). Notably, another

tubulin isotype, βIVb is shown to directly regulate EphrinB1 surface localization in oral cancer stem cells and their niche (5). Also, abrogation of βIVb tubulin or EphrinB1, which reduced the surface expression of EphrinB1 or the active EphrinB1 signaling, depleted the CSC population (5). Thus different tubulin isotypes play specific roles in the maintenance of CSCs by transporting unique signaling molecules involved in the regulation of stemness.

Modulation of Immune Evasion

In spite of the ability of immune cells to actively eliminate transformed cells, cancer cells survive in our body by manipulating the immune response machinery (92). This machinery, including CD4+ and CD8+ T cells, dendritic cells (DCs), and natural killer (NK) cells, is usually inhibited by certain checkpoint molecules, as a part of natural feedback inhibition, to prevent excessive immune reactivity (92). Cancer cells cleverly overexpress these checkpoint molecules including programmed death receptor ligands (PD-L1/PD-L2), and cytotoxic T cell-associated antigen-4 (CTLA-4) to evade the immune response (92). A growing body of evidence has demonstrated the active cross-talk of CSCs and immune infiltrates within the CSC niche (92). While the activity of some immune cells supports the expansion of CSCs, this subpopulation of cancer cells actively elicit immune evasion through a number of distinct mechanisms (92, 93). The metabolic reprogramming leading to the generation of CSCs can also regulate the immune evasion, as some of the metabolites produced by the alternative metabolism in the tumor microenvironment, like lactic acid and extracellular adenylate can regulate the fate of infiltrating immune cells (94). A more important aspect of metabolic reprogramming is the enhanced glycolytic activity-dependent up-regulation of PD-L1 that leads to the inhibition of cytotoxic T-cells (94). So tubulins as a mediator of metabolic reprogramming might regulate immune evasion also.

A recent study identifying immune related gene signatures in pancreatic cancer identified βIII tubulin as a critical immune regulator, closely linked to the T-cell receptor signaling pathway (95). This observation is in accordance with the earlier reports showing the importance of tubulin dynamics and molecular motors in immune synapse of T-cells and antigen presenting cells (96). Microtubule inhibitors, either anti-depolymerization agents such as the taxane family, or anti-polymerization agents such as colchicine and vinca alkaloids, have different effects on immune cell isotypes (97). Majority of the reports showing the effect of taxanes on immunemodulation attributes the activity of the drug on T-cells (97). Likewise, a widely used anti-polymerization agent, colchicine, down-regulates most immune cell types (97). Even though these reports showed the importance of tubulins and their cargos in T-cells for its function, there are some evidences to show that tubulins of the cancer cells, more specifically their inhibitors, can play a role in modulating immune response. One of the recent reports has shown that microtubule targeting agents, like vinca alkaloids and colchicine, can up-regulate the expression of PD-L1, a critical regulator of immune evasion (98). In clear cell renal cell carcinoma tissues the expression of βIII tubulin was associated with PD-L1 (39). Mechanistically, this correlation might be dependent on some of the tubulin interacting molecules. In agreement to the critical role of GLUT1 in glycolysis and metabolic reprogramming, the expression of PD-L1 is shown to depend on the activity of GLUT1 (99). Further, GRP78 is shown to physically interact with PD-L1 to increase its stability (100). Consistent with that, the enhanced expression of both PD-L1 and GRP78 is correlated with poor relapse-free survival in triple-negative breast cancer (100). As the different Ephrin ligands can engage the Eph receptors on immune cells and modulate their activity, Eph/Ephrin signaling is considered as a mediator of tumor immunity. More importantly, Eph/Ephrin signaling within the cancer cells can up-regulate the expression of PD-L1 (101). Since tubulins are shown to interact with these molecules, possibly regulating their localization and activity, specific tubulin isotypes present in the tumor microenvironment and/or CSC niche might be critical in the regulation of immune evasion.

CONCLUSION

Given the importance of the niche in the regulation of properties of stem cells or cancer stem cells, the mechanism involved in the generation of a niche is of prime importance. When we merge recent studies in the CSC field with tubulin research, a hypothesis of tubulin-mediated transport in defining stem cell niches emerge. Tubulins might be playing a role in the orchestrated expression of ligands and receptors to facilitate active signaling. On a broader concept, this transport mechanism might be critical in several scenarios, where coordinated localization of functional molecules is required. As this aspect of tubulins are poorly explored, extensive research on this is warranted to understand the mechanism behind establishing niches. In the cancer context, understanding the central players in defining CSCs has immense therapeutic potential. If we identify the specific isotype of tubulins responsible for defining CSC niche, strategies can be developed to target only those isotypes instead of inhibiting the whole cytoskeleton, which generally leads to chemoresistance.

AUTHOR CONTRIBUTIONS

TM conceived the idea and prepared the manuscript. DD contributed in the preparation of figures. SS contributed to the preparation of manuscript and critically modified it. All authors contributed to the article and approved the submitted version.

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