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Microtubules and their role in cellular stress in cancer

Amelia L. Parker*[1], *Maria Kavallaris*[1,2]* *and Joshua A. McCarroll*[1,2]

[1] *Tumour Biology and Targeting Program, Children's Cancer Institute Australia, Lowy Cancer Research Centre, University of New South Wales, Sydney, NSW, Australia*
[2] *Australian Centre for NanoMedicine, University of New South Wales, Sydney, NSW, Australia*

Edited by:
Megan Chircop, Children's Medical Research Institute, Australia

Reviewed by:
Matthias P. Wymann, University of Basel, Switzerland
Keith R. Laderoute, SRI International, USA

***Correspondence:**
Maria Kavallaris and Joshua A. McCarroll, Tumour Biology and Targeting Program, Children's Cancer Institute Australia, Lowy Cancer Research Centre, University of New South Wales, High Street, Randwick, Sydney, NSW 2052, Australia
e-mail: mkavallaris@ccia.unsw.edu.au; jmccarroll@ccia.unsw.edu.au

Microtubules are highly dynamic structures, which consist of α- and β-tubulin heterodimers, and are involved in cell movement, intracellular trafficking, and mitosis. In the context of cancer, the tubulin family of proteins is recognized as the target of the tubulin-binding chemotherapeutics, which suppress the dynamics of the mitotic spindle to cause mitotic arrest and cell death. Importantly, changes in microtubule stability and the expression of different tubulin isotypes as well as altered post-translational modifications have been reported for a range of cancers. These changes have been correlated with poor prognosis and chemotherapy resistance in solid and hematological cancers. However, the mechanisms underlying these observations have remained poorly understood. Emerging evidence suggests that tubulins and microtubule-associated proteins may play a role in a range of cellular stress responses, thus conferring survival advantage to cancer cells. This review will focus on the importance of the microtubule–protein network in regulating critical cellular processes in response to stress. Understanding the role of microtubules in this context may offer novel therapeutic approaches for the treatment of cancer.

Keywords: microtubules, tubulin, post-translational modifications, microtubule-associated proteins, stress response

INTRODUCTION

Microtubules, together with microfilaments and intermediate filaments, form the cell cytoskeleton. The microtubule network is recognized for its role in regulating cell growth and movement as well as key signaling events, which modulate fundamental cellular processes. Emerging evidence also suggests that it is critically involved in cell stress responses. This review will focus on the role of microtubules in this context in cancer.

Microtubules are composed of α- and β-tubulin heterodimers that associate to form hollow cylindrical structures (1) (**Figure 1**). They are highly dynamic, and are constantly lengthening and shortening throughout all phases of the cell cycle. During interphase, microtubules are nucleated at the centrosome (minus end) and radiate toward the cell periphery (plus end). Interphase microtubules are involved in the maintenance of cell shape and in the trafficking of proteins and organelles (1). Motor proteins translocate cell components on microtubule tracks, and protein–protein interactions with other adaptor proteins co-ordinate this process. Tubulin heterodimers also exist in soluble form in cells, and protein interactions with this tubulin population regulate microtubule behavior.

The addition and removal of soluble tubulin heterodimers to dynamic microtubule ends is a highly regulated process (**Figure 1**). Tubulin dimers are nucleotide binding proteins, with β-tubulin also possessing GTPase activity. The manner in which tubulin heterodimers are orientated in microtubules gives rise to a polar molecule that differs in both structure and kinetics at each end of the microtubule. The dynamics of tubulin addition and release are much slower at the minus end of the microtubule, which terminates with α-tubulin proteins, compared with the plus end of the microtubule, which terminates with β-tubulin proteins. The addition of a tubulin heterodimer to a microtubule activates the GTPase activity of β-tubulin, locking the β-tubulins in the microtubule in a GDP-bound state. The β-tubulins exposed to the solvent at the end of the microtubule form a GTP cap that is important in preventing microtubule depolymerization. Therefore, the binding of GTP at the microtubule plus end imparts structural and kinetic polarity to microtubules and is an important regulator of microtubule stability. It is believed that the polymerized and soluble tubulin pools interact with different signaling networks, however, the dynamic exchange of tubulin subunits between these pools makes it difficult to distinguish the functional roles of soluble and polymerized tubulin experimentally. The reader is referred to several excellent reviews for more detailed information on microtubule structure and dynamics (1, 2).

During mitosis, microtubules form the spindle to enable correct chromosomal segregation (3). Tubulin-binding agents (TBAs; e.g., taxanes, vinca alkaloids, epothilones, and eribulin) are important chemotherapeutic drugs that suppress spindle dynamics, causing subsequent mitotic arrest and cell death in rapidly dividing cells (3). Recent evidence suggests that the induction of cell stress in interphase cells also contributes significantly to TBA-mediated cell death (4–6), highlighting the importance of tubulin in cell stress responses in cancer.

In humans, microtubules are composed of combinations of eight α-tubulin isotypes and seven β-tubulin isotypes, with the different tubulin isotypes possessing specific tissue and developmental distributions (7) (**Table 1**). The members of the tubulin family share a high degree of structural homology and are distinguished from one another by highly divergent sequences at their carboxy-terminal (C-terminal) tail (8).

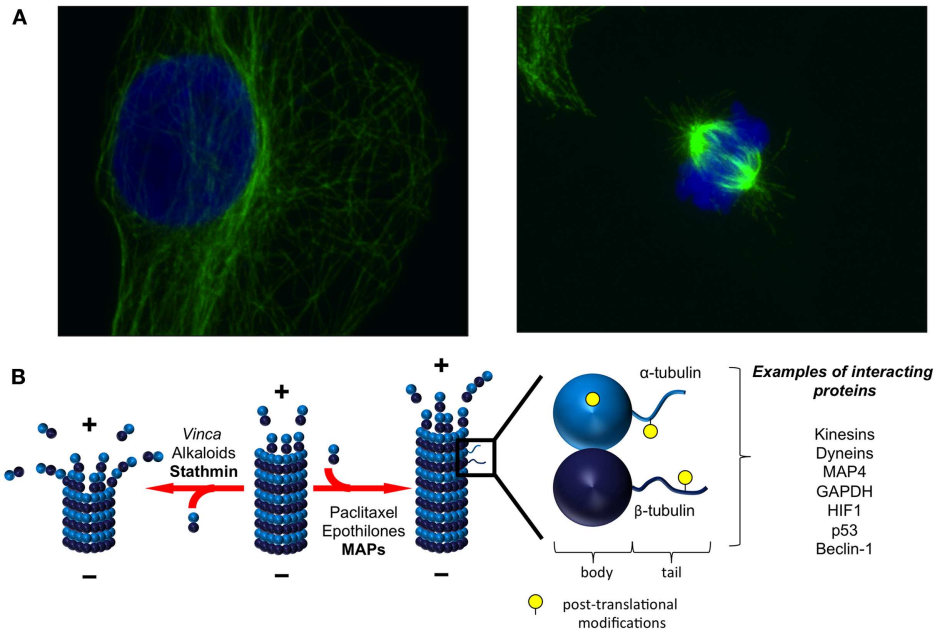

FIGURE 1 | Microtubules are dynamic structures that interact with diverse proteins. **(A)** Microtubules form a dynamic network and are constantly lengthening and shortening. In interphase [**(A)**, left], microtubules are anchored at the centrosome (minus end) and radiate toward the cell periphery (plus end). The microtubule network undergoes dramatic remodeling throughout the cell cycle, from interphase and through mitosis [**(A)**, right]. Green: α-tubulin, blue: DAPI. Images courtesy of Dr. Sela Po'uha. **(B)** Heterodimers of α- and β-tubulin associate to form microtubules. The dynamic addition and removal of tubulin heterodimers is faster at microtubule plus ends than at microtubule minus ends. Both endogenous factors and TBAs regulate and influence microtubule dynamics. A variety of proteins involved in cellular homeostatic mechanisms and stress responses also interact with tubulins either in their soluble or polymerized forms. Post-translational modifications on tubulins influence these interactions. Adapted with permission from Macmillan Publishers Ltd: Nature Reviews Molecular Cell Biology [Ref. (9)], Copyright 2011 and Nature Reviews Cancer [Ref. (15)], Copyright 2010.

Table 1 | Tubulin isotypes present in humans [Adapted with permission from Macmillan Publishers Ltd: Nature Reviews Cancer (Ref. (15)) Copyright 2010 and Elsevier (Ref. (233)) Copyright 2009].

Tubulin isotype	Gene name	Accession number
α-TUBULIN		
α1A-Tubulin	TUBA1A	NP_006000
α1B-Tubulin	TUBA1B	AAC31959
α1C-Tubulin	TUBA1C	Q9BQE3
α3C-Tubulin	TUBA3C	Q13748
α3E-Tubulin	TUBA3E	NP_997195
α4A-Tubulin	TUBA4A	NP_005991
α8-Tubulin	TUBA8	Q9NY65
α-Like 3-Tubulin	TUBAL3	NP_079079
β-TUBULIN		
βI-Tubulin	TUBB	NM_178014
βII-Tubulin	TUBB2A, TUBB2B	NM_001069; NM_178012
βIII-Tubulin	TUBB3	NM_006086
βIVa-Tubulin	TUBB4	NM_006087
βIVb-Tubulin	TUBB2C	NM_006088
βV-Tubulin	TUBB6	NM_032525
βVI-Tubulin	TUBB1	NM_030773

The authors direct readers to comprehensive reviews (233) for further information on tubulin isotype structure.

The C-terminal tails of tubulin are also thought to mediate protein–protein interactions and act as sites of post-translational modifications to confer unique functionality to each isotype (9).

TUBULIN ALTERATIONS IN CANCER

Diverse changes in the microtubule network have been identified and characterized in a wide variety of cancers, including altered expression of tubulin isotypes, alterations in tubulin post-translational modifications, and changes in the expression of microtubule-associated proteins (MAPs) (**Table 2**). Despite evidence from *in vitro* studies associating tubulin mutations with resistance to TBAs (10–13), tubulin mutations are not clinically prevalent and their importance in disease progression and chemotherapy resistance is controversial (14). Microtubule alterations are thought to influence cellular responses to chemotherapeutic and microenvironmental stressors, thereby contributing to broad spectrum chemotherapy resistance, tumor development, and cell survival.

CHANGES IN TUBULIN ISOTYPE COMPOSITION

Altered tubulin isotype expression is the most widely characterized microtubule alteration reported in cancer and has been observed in both solid and hematological tumors. These changes are often associated with chemotherapy resistance and poor

Table 2 | Clinical studies of tubulin alterations in cancer.

Microtubule alteration	Observation	Effect	Cancer	Reference
Altered isotype expression	High βI-tubulin	Poor response to docetaxel treatment	Breast cancer	(234)
	High βIII-tubulin expression	Poor survival, poor outcome for surgical resection or TBA response; correlates with subtype	Non-small cell lung cancer (NSCLC)	(21, 31, 108, 235–238)
		Correlates with poor survival, poor response to platinum and taxane treatment, advanced stage, or aggressive disease	Ovarian cancer	(13, 16, 35, 239–242)
		Favorable response to taxane treatment	Ovarian (clear cell adenocarcinoma)	(243)
		Poor response to taxane treatment	Breast cancer	(234, 244)
		Correlates with disease stage	Pancreatic ductal adenocarcinoma	(17)
		Correlates with disease stage	Glioblastoma	(101)
		Localized to invasive edge	Colorectal cancer	(245)
		Poor response to taxane/platinum treatment	Uterine serous carcinoma	(246)
		Poor response to taxane treatment	Gastric cancer	(247)
		Aggressive disease, patient outcome	Prostate cancer	(36, 248, 249)
	Low βII-tubulin expression	Correlates with poor response to taxane treatment or advanced stage disease	Breast and ovarian cancer	(239, 250)
	High βIVa-tubulin expression	Poor response to taxol treatment	Ovarian cancer	(240)
	High βV-tubulin expression	Favorable response to taxane treatment	NSCLC	(251)
	High α1b-tubulin expression	Histological grade	Hepatocellular carcinoma	(252)
	High γ-tubulin expression	Poorly differentiated	Medulloblastoma	(253)
Altered post-translational modification	High Δ2α-tubulin	Poor response to vinca alkaloid treatment	Advanced NSCLC	(238)
	High detyrosinated tubulin	Disease aggressiveness	Breast cancer	(48)
	Active tyrosination cycle	Favorable patient outcome	Neuroblastoma	(50)

prognosis (**Table 2**) [reviewed in Ref. (15)]. Compared with α-tubulin isotypes, β-tubulin isotypes have received more attention in this context, largely due to the availability of isotype-specific antibodies, and the fact that TBAs bind to the β-tubulin subunit to exert their toxic effect. Furthermore, βIII-tubulin is the most comprehensively examined isotype across a variety of cancers.

Elevated βIII-tubulin levels are associated with poor prognosis in a host of different epithelial cancers. In addition to TBA resistance, βIII-tubulin levels influence sensitivity to non-tubulin-targeted agents [reviewed in Ref. (15)]. The clinical observations are supported by numerous *in vitro* studies where altered βIII-tubulin levels confer resistance to a broad spectrum of drug classes in solid and hematological tumors [reviewed in Ref. (15)]. Coupled with evidence that βIII-tubulin is also involved in tumor development and disease aggressiveness (16–18), these results suggest that βIII-tubulin may be acting as a survival factor in cancer.

Altered levels of βII-, βIVa-, βIVb-, and βV-tubulins have also been associated with resistance to TBAs in a number of drug resistant cancer cell types (19–26). However, the clinical relevance of these specific tubulin isotypes is limited and requires further investigation. Moreover, the involvement of tubulin isotypes in disease progression is complex, and depends on both the treatment regime and disease stage (27). Additional complexity may be conferred by interactions between different isotypes, since the overexpression of specific β-tubulin isotypes, such as βI, βII, and βIVb, does not affect TBA resistance in Chinese Hamster Ovary cells (28, 29). For βIII-tubulin the results have been conflicting. Overexpression of βIII-tubulin failed to confer resistance to TBAs in prostate cancer (28, 29). In contrast, overexpressing this isotype in Chinese Hamster Ovary cells conferred resistance to paclitaxel (30).

In cancer, alterations in the tubulin isotype composition have been detected at both the gene and protein level and result from increased gene transcription and enhanced mRNA stability (24). However, tubulin mRNA levels do not always reflect protein expression due to the complexity of post-translational mechanisms that control tubulin expression (24, 31). For instance, the tumor suppressor miR-100 and the miR-200 family of microRNAs (24, 32, 33) as well as epigenetic mechanisms (34, 35) are implicated in coordinating β-tubulin isotype expression. Therefore, dysregulation of miRNA networks and epigenetic mechanisms in cancer may also contribute to aberrant tubulin isotype expression in cancer. Recent evidence showing an association between elevated βIII-tubulin expression and PTEN deletions in prostate cancer also suggest that changes in the levels of this isotype may result from PTEN-mediated genetic reprograming (36).

Cell stress influences the tubulin isotype composition. For example, βIII-tubulin expression can be induced (24, 37) or decreased (16) by chemotherapy treatment. The induction of βIII-tubulin has been observed in response to vinca alkaloid treatment in breast cancer cells through an activator protein-1 (AP-1) site on the βIII-tubulin promoter (38), while its induction in hypoxic and hypoglycemic conditions in ovarian cancer cells is mediated by hypoxia-inducible factor (HIF) 1α and Hu antigen (HuR), respectively, at the 3′ untranslated region (UTR) (39, 40). The latter mechanism is a regulatory feature commonly utilized by proteins involved in cell stress, and enables rapid changes in protein levels (41). However, it is to be noted that the regulation of βIII-tubulin levels in cell stress responses may depend upon the basal expression of the protein and may also be cell type specific.

Initially, differences in the drug binding affinity and structural characteristics of microtubules composed of different β-tubulin isotypes were thought to explain correlations between aberrant tubulin isotype compositions and resistance to TBAs. However, recent observations correlating changes in isotype expression with tumor development and resistance to non-TBA agents have challenged the simplicity of this model. With increased recognition of the importance of cell stress responses in chemotherapy efficacy, isotype-mediated modulation of these responses may contribute to chemotherapy resistance. In particular, cellular homeostasis relies on a dynamic microtubule network and may be perturbed by alterations in microtubule stability and dynamics. The microtubule isotype composition does affect microtubule stability, with consequences for TBA sensitivity (7, 23, 42). Stable microtubules play an important role in cellular trafficking and their role in multiple stress responses are discussed below. Chemotherapy agents that do not bind to tubulin can also affect microtubule stability by unknown mechanisms (43), and this may represent a mechanism common to chemotherapy agents of different classes.

The tubulin isotype composition can also influence microtubule dynamics. In non-small cell lung cancer (NSCLC) cells, suppression of βIII-tubulin using RNA interference technology decreases microtubule dynamics in the presence of TBAs, but has no effect under basal conditions (44). These observations suggest that changes in isotype composition may influence microtubule dynamics in the presence of chemotherapeutic stressors but not under basal conditions; however, a direct causal relationship between isotype expression, microtubule dynamics, and cell survival in response to these and other stressors has not been established. In general, the importance of microtubule dynamics in homeostatic cell signaling suggests that cell stress responses, and not just spindle dynamics, may be impacted by aberrant isotype expression in cancer, thus offering an additional determinant of chemosensitivity.

TUBULIN POST-TRANSLATIONAL MODIFICATIONS

Tubulins are subject to diverse post-translational modifications (PTMs) [reviewed in Ref. (9)]. The majority of tubulin PTMs are highly heterogeneous, and little is understood about the regulation and impact of these modifications. Post-translational modifications are thought to regulate protein–protein interactions with the microtubule cytoskeleton, thereby affecting signaling events within the cell. The majority of these modifications are localized to the tubulin C-terminus and potentially impart specific functions to the different tubulin isotypes.

Removal and addition of the α-tubulin C-terminal tyrosine occurs cyclically in cells. Tyrosine addition and removal are catalyzed by tubulin tyrosine ligase (TTL), and carboxypeptidases, respectively (9). Highly dynamic microtubules are more likely to be detyrosinated, due to the kinetic balance between higher TTL and carboxypeptidase activities on the soluble and polymerized tubulin pools, respectively (45). While traditionally viewed as an intrinsic hallmark of stable microtubules, the detyrosination motif alters motor protein recruitment to microtubules, thereby stabilizing microtubules and influencing trafficking functions within the cell (46).

Tyrosination modifications of α-tubulin are known to be critical for differentiation, cell cycle progression, organelle trafficking, and vesicular transport (9). Altered levels of tyrosination modifications and the enzymes responsible for them have been detected in a range of cancers and are associated with more aggressive disease (47–50). For instance, loss of TTL induces mesenchymal transition in breast cancer cells, which may contribute to increased metastatic potential and altered cell stress responses (51).

Increased acetylation of α-tubulin on Lys40 has also been observed in tumor cells (52). Elevated HDAC6 expression, one of several regulators of tubulin acetylation, is associated with better prognosis in breast cancer (53). Sirtuin-2 is also responsible for tubulin deacetylation (54) and has been linked with the regulation of autophagy in response to stress [reviewed in

Ref. (55)]. HDAC6 does influence microtubule stability (56), however, whether acetylation itself influences microtubule stability remains uncertain. Acetylated tubulin is implicated in intracellular trafficking (57), endoplasmic reticulum (ER) localization, and ER–mitochondria interactions (58), as well as the regulation of microtubule dynamics (59). The involvement of α-tubulin acetylation in a broad range of cell functions may reflect its importance in the maintenance of cellular homeostasis.

Other post-translational modifications have been detected in prostate and hepatic cancers. Removal of the final two residues of the βIVb-tubulin C-terminal tail was identified in higher stage liver cancer and in a mouse model of hepatic carcinoma (60). Polyglutamylated α-tubulins (47) and the polyglutamylation enzyme TTL-like 12 are elevated in prostate cancer and correlate with more aggressive disease (61).

Overall, despite a lack of clarity surrounding the mechanistic details of the function of tubulin PTMs, mounting evidence points to their role in fundamental cell processes. The diverse PTM alterations observed in a range of cancers are likely to perturb homeostatic processes, thereby contributing to stress response signaling. Detailed spatiotemporal mapping of tubulin PTMs and proteomic studies investigating their role in signaling networks are required to elucidate the influence of tubulin PTMs on cellular stress responses.

MICROTUBULE-ASSOCIATED PROTEINS

A wide variety of proteins are known to interact with tubulins. Interactions between tubulin and MAPs influence microtubule stability and dynamics, and are known to affect chemotherapy sensitivity and tumor growth in cancer [reviewed in Ref. (62)]. Aberrant expression of primarily neuronal MAPs (e.g., Tau, MAP2) has been detected in non-neuronal cancer tissue. For example, tau overexpression is correlated with poor outcome in breast cancer, and this protein may influence taxane sensitivity by decreasing the affinity of the drug for β-tubulin (63). Altered MAP2 expression is also associated with taxane resistance (22, 64), with differential effects in primary and metastatic melanoma (65).

Increased MAP4 expression and altered expression of multiple MAP4 isoforms have been detected in TBA-resistant leukemia and NSCLC cells *in vitro* (10, 11, 66). In addition, changes in stathmin, survivin, BRCA1, CLIP170, and VHL expression have all been associated with chemotherapy resistance and disease progression (62, 67). For instance, stathmin was recently shown to play an important role in regulating neuroblastoma cell migration and invasion (68). Moreover, silencing its expression using RNAi gene-silencing technology significantly reduced lung metastases in a clinically relevant orthotopic neuroblastoma mouse model (68). The overexpression of kinesins also influences chemotherapy sensitivity and disease progression through mitotic and non-mitotic mechanisms [reviewed in Ref. (69)]. A recent study has shown that kinesins interact differentially and specifically with tubulin isotypes and tubulin post-translational modifications (70). In this way, changes in tubulin isotype expression and post-translational modifications seen in cancer may also influence motor protein function and the numerous basic processes that depend upon these interactions.

The effect of MAPs on cell function in cancer is complex, with interactions between individual MAPs influencing survival and metastases. Progress toward understanding the functional consequences of these proteins and their signaling networks in cancer relies upon more comprehensive characterization of the interactions between tubulins and MAPs, and the influence of tubulin isotypes and PTMs on these interactions.

MICROTUBULE CYTOSKELETON IN STRESS RESPONSES

Microtubules influence homeostatic mechanisms and cell stress responses by regulating intracellular trafficking, acting as a scaffold for the co-localization and sequestration of stress response proteins, transmitting stress signals through cytoskeletal remodeling and modulating the induction of cell death pathways. Examples of their role in these processes are described below.

MICROTUBULES AND CELLULAR SIGNALING

While microtubules possess distinct functions in particular stress responses, the microtubule network also influences common signaling pathways engaged by a variety of cellular stresses. Stress response signaling requires trafficking of proteins and organelles throughout the cell and modulation of the microtubule network is expected to influence signal transduction events. For example, TBAs differentially suppress microtubule-mediated intracellular transport in neuronal cells (71).

In addition to general effects on signal transduction, microtubules regulate mitogen activated protein kinase (MAPK) signaling. The MAPK superfamily includes extracellular regulated kinases (ERK), c-Jun N-terminal protein kinase (JNK), and p38 families and is critically involved in mediating the initiation and execution of a range of cellular stress responses [reviewed in Ref. (72)]. MAPK proteins interact extensively with the microtubule network, with one-third of the total MAPKs associating with microtubules through kinesin motor proteins (73). Interactions between microtubules and these signaling proteins can regulate and co-ordinate widespread cellular stress signaling events.

The JNK signaling pathway is induced by a wide range of environmental stressors (72) and TBAs activate this pathway in the induction of apoptosis (74–76). In particular, JNK signaling is required for the execution of apoptosis in response to ER stress and autophagy (77). JNK co-ordinates cytoskeletal architecture in normal cells and JNK1 regulates microtubule dynamics (78, 79). JNK1 also phosphorylates MAP1 and MAP2 to alter their distribution and microtubule architecture (79). In this context, JNK, the heavy chain kinesin family-5B protein and βIII-tubulin form a complex, raising the possibility that alterations in β-tubulin isotype composition may affect JNK pathway activation and cell death responses.

While TBAs generally activate JNK signaling to initiate apoptosis [reviewed in Ref. (80)], microtubule stabilizing and destabilizing agents differentially influence downstream signaling events, suggesting that microtubule stability regulates JNK signaling (81). Compared with etoposide and doxorubicin, vinblastine uniquely causes c-Jun phosphorylation, AP-1 activation, ERK inactivation, and p53 downregulation (81). Microtubule destabilizing and stabilizing agents initiate apoptosis via JNK signaling through AP-1 dependent and AP-1 independent mechanisms, respectively

(82). The AP-1 dependent pathway leads to positive feedback of c-Jun levels and sustained JNK signaling (82), suggesting that microtubule–JNK interactions may constitute a feedback loop for the amplification and damping of signaling pathways to regulate stress response kinetics.

Extracellular regulated kinase also interacts with microtubules and phosphorylates MAPs to regulate their activity (83, 84). MAPK-mediated MAP phosphorylation is implicated in hypoxic stress responses (85). Differential induction of ERK signaling by TBAs may also mediate downstream effects independently of apoptosis induction (86).

It is well established that microtubules are involved in the translocation of messenger proteins between different cell compartments to enable efficient signal transduction. However, increasing evidence supports a role for microtubule dynamics, tubulin isotypes, and MAPs in specifically regulating the course, amplitude, and kinetics of MAPK signaling.

p53 AND MICROTUBULES

p53 is a key mediator of cellular stress responses and its activity heavily depends on microtubules (87). p53 is translocated to the nucleus along microtubule tracks by dynein proteins in a complex with heat shock protein 90 (Hsp90) and Hsp90 immunophilins (87–89). The binding of Hsp90 to p53 inhibits MDM2-mediated degradation of the protein by the ubiquitin–proteasome system (90).

Microtubule dynamics regulate p53 levels. p53 levels and its nuclear accumulation are increased by TBA treatment at doses that suppress microtubule dynamics but do not disrupt the structure of the microtubule network (87, 91). MAP1B also associates with p53, decreasing its activity and inhibiting doxorubicin-induced apoptosis in neuroblastoma cells (92). p53 signaling can influence microtubule dynamics and remodeling, as well as the expression of tubulin isotypes and MAPs (93). Taken together, by regulating p53 levels and translocation, microtubules significantly impact p53-mediated stress response signaling.

HYPOXIA

Rapid cell proliferation and poor vascular development leads to hypoxic regions within solid tumors. Hypoxia-inducible factor 1 (HIF1) is considered to be the master regulator of cellular adaptation to hypoxia and is upregulated in a large proportion of solid cancers (94).

In the absence of oxygen, HIF1α heterodimerizes to the constitutively active β subunit to initiate transcriptional changes [reviewed in Ref. (95)]. HIF1α stabilization is regulated by enzyme-mediated hydroxylation, which enables recognition of HIF1α for ubiquitinylation and degradation by proteins such as the von Hippel–Lindau (VHL) protein (96). Low oxygen levels inactivate the hydroxylases, leading to stabilization and nuclear translocation of the α subunit where the HIF1 heterodimer binds to hypoxia responsive elements in target gene promoters (95).

Dramatic microtubule remodeling occurs under hypoxic conditions. Decreased microtubule polymerization has been observed in response to anoxic conditions (0–2% O_2) (85, 97), while increased microtubule polymerization has been observed in physiological hypoxia (3% O_2) (98). Enhanced microtubule polymerization under these conditions is coupled with increased tubulin detyrosination and glycogen synthase kinase 3β (GSK3β) inhibition (98), while phosphorylation of the MAPs dynein light chain tctex-type 1 (DYNLT1), MAP4, and stathmin have each been associated with microtubule depolymerization (85). Discrepancies between these observations may be due to the differential effects of anoxia compared with physiological hypoxia, or alternatively may reflect the role of the GSK3β pathway and MAP interactions on microtubule remodeling (98). Hypoxic activation of the p38/MAPK pathway contributes to phosphorylation of MAP4 and stathmin (85). Microtubule remodeling in response to hypoxia may impact metastatic processes with increased microtubule polymerization influencing integrin trafficking and invasion in breast cancer cells (98).

MAP4 protects against microtubule disruption during hypoxia by enhancing tubulin polymerization and concomitant upregulation of tubulin expression (97). It also maintains ATP production under hypoxic conditions and prevents mitochondrial permeabilization (97). The non-phosphorylated form of DYNLT1 also protects against microtubule disruption and mitochondrial permeabilization and maintains the cellular energy status in hypoxia, with phosphorylation of DYNLT1 potentiating cell death through mitochondrial permeabilization (99). DYNLT1-mediated interactions between tubulin and Voltage Dependent Anion Channels (VDACs) may facilitate cross-talk between the microtubule cytoskeleton, intrinsic apoptotic pathway, and mitochondrial quality control system to influence cell survival in hypoxia (97).

Hypoxic adaptation may also be regulated by specific tubulin isotypes in cancer cells. For instance, βIII-tubulin (encoded by the TUBB3 gene) is induced under hypoxic conditions by direct binding of HIF1α to the E box motif within its 3′UTR (39). Hypoxic upregulation of this isotype appears to be cell type specific, depends on the epigenetic status of the TUBB3 3′UTR and is also influenced by the basal βIII-tubulin expression level (26, 39). The expression of this tubulin isotype is also regulated by HuR (40), which is involved in HIF1α stabilization (100). High βIII-tubulin expression is also detected in close proximity to necrotic tumor regions, further supporting a role for this protein in hypoxic adaptation (101).

Hypoxia-inducible factor 1α degradation is dependent on the short isoform of VHL, while the long isoform is a known regulator of microtubule dynamics (102). In renal cell carcinoma, where VHL mutations result in upregulated HIF expression, there is a loss of microtubule–HIF coupling, suggesting that VHL may be responsible for microtubule-mediated regulation of HIF signaling (103). However, the mechanisms underlying this observation and the functional consequences of this regulatory process are uncertain.

Hypoxia-inducible factor 1 activity depends upon its ability to translocate to the nucleus, and microtubules act as tracks for dynein-mediated HIF1 translocation (103). Suppression of microtubule dynamics decreases HIF1α levels by increasing HIF1α mRNA association with inactive ribosomal subunits and by targeting this mRNA to P-body components (104). Suppression of microtubule dynamics and HIF nuclear translocation prevents VEGF-mediated hypoxic adaptation in prostate and breast

cancer cells and decreases angiogenesis in a murine orthotopic breast tumor model (94). However in this study, microtubule dynamics regulated HIF1α levels to the same extent in both normoxic and hypoxic conditions; therefore, this mechanism may not be responsible for regulating HIF1α levels specifically in response to hypoxia. Recent evidence suggests that hypoxic adaptation also depends upon microtubule-mediated perinuclear mitochondrial clustering (105), and highlights the importance of organelle localization in cellular adaptation to hypoxia.

Overall, the hypoxic response is associated with dramatic microtubule remodeling, and altered MAP signaling to maintain bioenergetics and organelle function under hypoxic conditions. The microtubule network also regulates hypoxic adaptation by affecting HIF1α signaling and organelle localization, placing microtubules as a central player in the hypoxic stress response. While current evidence suggests that β-tubulins may function in an isotype-specific manner in this context, a more comprehensive analysis of the contributions of each individual isotype to hypoxic adaptation is required.

OXIDATIVE STRESS

Aberrant oxidative stress signaling has been reported in many cancers. The upregulation of enzymes responsible for redox homeostasis, metabolic reprograming, and exposure to extracellular inducers of intracellular oxidative species all contribute to aberrant oxidative conditions in cancer [reviewed in Ref. (106)]. Markers of oxidative stress correlate with chemotherapy response and upregulation of redox enzymes, such as glutathione peroxidases, have been observed in the acquisition of chemotherapy resistance and genomic instability [reviewed in Ref. (106)].

Tubulins interact with mediators of the oxidative stress response, with direct interactions between βIII-tubulin and glutathione *S*-transferase μ4 observed in ovarian cancer cells (107). βIII-tubulin and the DNA damage repair enzyme excision repair cross-complementation group-1 (ERCC1) act together to influence patient response to taxane and paclitaxel combination treatment (108); however, the mechanisms underlying this co-operative effect are unknown.

Specific tubulin isotypes may also alter oxidative stress responses by acting as redox switches (109). In particular, ser/ala124, which is a cysteine in βIII-, βV- and βVI-tubulins, and cys239, which is a serine in βIII-, βV-, and βVI-tubulins, have been specifically identified as potential sensors of oxidative stress (109). Cys239 is readily oxidized and its oxidation inhibits microtubule assembly and stability (109). Therefore, alterations in tubulin isotype composition may influence microtubule stability in an oxidative environment to maintain microtubule integrity and cell survival in these adverse conditions. Moreover, oxidative stress influences tubulin post-translational modifications. Nitrotyrosine is a common byproduct of nitrosyl radical production in oxidative stress and can be incorporated into microtubules through the tyrosination/detyrosination cycle (110). While nitrotyrosine incorporation does not affect microtubule assembly, architecture, or cell viability (111), it does increase the stability of neuronal microtubules (112). Furthermore, elevated levels of nitrosylated α-tubulins correlate with disease stage in gliomas (113).

Oxidative stress is also induced by TBAs, suggesting an involvement of microtubules in oxidative stress responses, and is an important mechanism of action for platinum-based chemotherapeutic agents (114). Paclitaxel treatment induces reactive oxygen species through activation of the JNK pathway in melanoma cells (115). TBA treatment also influences NADPH oxidase activity, increases ROS levels and induces bystander effects in breast cancer cells (116). This effect may be mediated by changes in microtubule dynamics and stability, with these factors regulating Rac1 translocation and subsequently, NADPH oxidase activity (117, 118).

Studies in neurons and endothelial cells indicate that the microtubule cytoskeleton undergoes remodeling in response to oxidative stress (119). Oxidative stress induces microtubule depolymerization, and increases the pool of soluble tubulin (120, 121). 4-Hydroxy-2-nonenal (4-HNE), a secondary product of lipid peroxidation and marker of oxidative stress, also causes microtubule depolymerization, together with tubulin crosslinking (122, 123). This depolymerization may be caused by preferential reaction of 4-HNE with soluble tubulin, thereby disrupting the soluble/polymer fractionation of tubulin subunits and subsequent microtubule assembly (124). Interactions between microtubules and MAPs protect microtubules from depolymerization in response to oxidative stress (122, 125), and alters cellular trafficking in oxidative conditions (126).

Collectively, there is growing evidence supporting a role for tubulin isotypes and the microtubule network in both sensing and responding to oxidative stress in cancer through direct structural changes and protein–protein interactions. This is supported by observations in neuronal models, however, the specific roles of tubulin isotypes and their accessory proteins in oxidative stress responses remain to be clarified.

METABOLIC STRESS

Metabolic stress occurs in cancer as a result of uncontrolled cell proliferation in the absence of adequate nutrients [reviewed in Ref. (127)]. Microtubules and tubulins are involved in responding to metabolic stress by sensing and modulating metabolic processes to maintain cellular energy levels. The microtubule network is hypothesized to play a critical role in the regulation of cellular metabolism (128).

Early studies suggested that microtubules may act as a sensor of the energy state of the cell (129) with ATP depletion causing instability of detyrosinated microtubule plus ends (130, 131). AMPK is a major sensor for the metabolic state of the cell and affects microtubule dynamics by phosphorylation of CLIP170 (132). CLIP170 alters paclitaxel sensitivity in breast cancer cells by enhancing the binding of the drug to tubulin (67). In neuronal cells, activation of AMPK in metabolic stress prevents growth of axonal microtubules (133), further supporting a role for microtubules in early metabolic stress signaling events. The main neuronal tubulin, βII-tubulin, was also identified as a downstream target of AMPK in murine brain extracts (134).

Metabolic modulation of microtubule dynamics and tubulin post-translational modifications may allow for rapid and widespread stress responses. For example, nutrient starvation induces hyperacetylation of tubulin, which may act in concert with

AMPK to induce autophagy in response to decreased ATP levels (77), thereby engaging multiple stress response pathways through microtubule-related signaling.

METABOLIC REGULATION

Tubulins and microtubules have been suspected to function as a key modulator of mitochondrial metabolism for some time (128). Recent studies have demonstrated that tubulin is capable of interacting with, and blocking the VDAC, thereby regulating ATP and metabolite compartmentalization and contributing to the Warburg effect (135–138). This interaction is mediated by the tubulin C-terminal tail (135), raising the possibility that post-translational modifications and different tubulin isotypes may differentially regulate VDAC dynamics to influence metabolic reprograming in cancer.

Tubulins, and in particular βIII-tubulin, associate with enzymes of the tricarboxylic acid cycle and glycolysis (107). *In vitro* studies in reduced systems showed that tubulin interacts with a variety of glycolytic enzymes including pyruvate kinase, phosphofructokinase, aldolase, hexokinase, GAPDH, and lactate dehydrogenase (139–144). Interactions with some of these enzymes may be isotype-specific, by interacting with the α-tubulin C-terminal tail (142) rather than the tubulin body (140).

Preferential interactions between glycolytic enzymes and either the soluble or polymerized tubulin pool may also influence metabolic activity and microtubule dynamics (139, 141, 144). GAPDH activity is differentially regulated by its interaction with either the soluble or polymerized tubulins (143), and this interaction influences microtubule dynamics (145). Interactions between metabolic enzymes and tubulins may therefore mediate bi-directional signaling events to sense and respond to metabolic stress. Indeed, mathematical modeling of metabolic pathways and tubulin's modulation of enzyme activity suggest that glycolytic flux is regulated by microtubule polymer levels (146), however, the mechanisms by which the microtubule network influences metabolic homeostasis and the importance of the soluble and polymerized tubulin fractions in these functions remain to be characterized experimentally.

The association between GAPDH and microtubules may also influence cellular trafficking, with a recent study finding that ATP generated from vesicular GAPDH activity fuels the energy consumption of motor proteins during vesicular transport (147). Furthermore, GAPDH is known to mediate membrane fusion, and its association with microtubules may co-regulate membrane trafficking during glycolytic stress (148). The presence of GAPDH on microtubules allows the recruitment of Rab2 protein to regulate membrane and ER–Golgi trafficking independently of its catalytic activity (145, 149). Given the importance of ER–Golgi trafficking in protein glycosylation, the interaction of GAPDH with microtubules may function as a point of communication between metabolic and protein modification pathways under a range of stresses. For example, in neuronal cells, GAPDH binds tubulin through the neuronal MAP1B protein but is relocalized upon oxidative stress (150).

Specific interactions between tubulin isotypes and glycolytic enzymes support the pro-survival effect of altered tubulin isotypes in cancer. Pyruvate kinase interacts with tubulin via the tubulin C-terminal tail and depolymerizes stabilized microtubules (140, 151). In particular, βIII-tubulin interacts with the mitochondrial-localized pyruvate kinase M2 (107), which is associated with the Warburg effect. Feedback from metabolic products also influences the association of pyruvate kinase with microtubules, as well as microtubule stability (151), further supporting a role for the microtubule cytoskeleton in the regulation of metabolic flux. Altered metabolic activity also influences microtubule architecture (152), raising the possibility that the microtubule system may communicate with metabolic networks in a bi-directional manner.

βIII-tubulin has been specifically implicated in glucose stress responses. Treatment of ovarian cancer cells with tunicamycin or wortmannin to block protein glycosylation and PI3K signaling, respectively, upregulates βIII-tubulin and alters the post-translational modifications of non-mitochondrial tubulins in cell lines with low basal βIII-tubulin expression (107). βIII-tubulin induction and decreased βI-tubulin expression have also been observed for ovarian cancer cells under glucose starvation (40). Upregulation of βIII-tubulin in these conditions correlates with HuR binding to the βIII-tubulin 3′UTR (40). This function of HuR is independent of its role in the nuclear export of mRNA; however, whether HuR is involved in the stabilization of βIII-tubulin mRNA under hypoglycemic conditions was not investigated. Correlations between increased HuR, βIII-tubulin expression, and poor survival in ovarian cancer samples further support a role for this mechanism in influencing cancer progression and patient outcome (40).

The current evidence strongly supports a role for the microtubules in regulating metabolic activity and metabolic reprograming in response to nutrient starvation. However, the mechanistic details underpinning these observations is lacking and the importance of specific tubulin isotypes, tubulin post-translational modifications, and associated proteins in regulating metabolic stress responses requires further characterization.

AUTOPHAGY

Macroautophagy (hereafter referred to as autophagy) can be induced in cells in response to diverse stresses, including metabolic and ER stress [reviewed in Ref. (153)]. Autophagy is a catabolic process that enables isolation and recycling of protein and organelle components by sequestering them into vacuoles for subsequent lysosomal degradation (154). It is also an important quality control process, allowing for the removal of damaged organelles and proteins, and protects cells from oxidative stress damage (155). Autophagic activity can support cells during ATP depletion, and thus is intrinsically linked with metabolic stress responses (154).

Recent evidence supports a role for autophagy in the survival and treatment sensitivity of cancer cells, and several recent reviews have been devoted to this topic (156–158). Microtubules have been known to play a critical role in autophagic flux for several decades (159), however our understanding of their importance in autophagy initiation, trafficking, and lysosomal fusion has been furthered in recent years.

Evidence for a microtubule role in autophagy regulation comes from the alteration of autophagic flux upon treatment with TBAs *in vitro* (160–163). Disruption of autophagic flux by

TBAs is important in the mechanism of action of, and resistance to, TBAs in cancer (4, 164). The influence of TBAs on autophagy may be mediated by inhibition of Akt/mammalian target of rapamycin (mTOR) signaling (165), or suppression of microtubule dynamics, and additional studies are required to characterize this mechanism.

Microtubule-associated protein-1 light chain 3 (MAP1LC3, also referred to as LC3), a critical member of the autophagy network, interacts directly with tubulin in both its free and phosphatidylethanolamine-conjugated form (77, 160). LC3 also interacts with microtubules through MAP1 proteins (166–168). The promotion of autophagy by MAP1S reduces genomic instability to suppress tumor development in hepatocarcinoma, and MAP1S may also co-ordinate mitochondrial dynamics and autophagy (155, 167). Other autophagy proteins also associate with microtubules, including ULK1, Beclin-1, WIPI1, autophagy related (Atg) protein 5, and Atg12, which are thought to be principally involved in autophagosome formation (77, 169, 170). In neuronal models derived from neuroblastoma cells, autophagy inhibition is associated with decreased β-tubulin levels and suppressed neurite outgrowth (171). However, links between altered tubulin expression and autophagy have not yet been reported in non-neuronal cancer cells.

Autophagy initiation involves activation of the master regulator mTOR and the formation of the mTOR-containing complexes. mTOR activity is regulated by lysosomal localization (172), with mTOR associating specifically with peripheral lysosomes (173). Peripherally localized mTOR is sensitive to nutrient starvation, which causes it to be released from lysosomes to form the mTORC1 complex and initiate autophagy (172). Microtubules control the peripheral localization of lysosomes, and therefore ensure the sensitivity of mTOR to nutrient starvation (172). Spatial partitioning of the microtubule-interacting kinesins KIF2A and KIF1B between peripheral or perinuclear lysosomes also influences mTOR activation and the initiation of autophagy (173).

Microtubules act as scaffolds and sequester proteins to regulate autophagy. Activating molecule in BECN1-regulated autophagy 1 (AMBRA1) acts as a linker protein between microtubules and the PI3K signaling complex responsible for autophagy induction (169). Starvation induces phosphorylation of AMBRA1 by ULK1, releasing the Beclin-1-PI3K complex from microtubules to the ER to initiate autophagosome formation (169). Beclin-1–Bcl-2 complexes are also sequestered on microtubules during periods of high nutrient availability. JNK1-mediated phosphorylation of Bcl-2 in response to nutrient starvation causes dissociation of Beclin-1 from this complex to initiate autophagosome signaling and influence apoptosis (174). Microtubules are also involved in the transport of several proteins whose localization is required for autophagosome formation (175).

Tubulin post-translational modifications also regulate autophagy initiation, as tubulin hyperacetylation occurs before autophagosome formation in response to nutrient starvation (77). Acetylation modifications signal kinesin recruitment to microtubules, with subsequent JNK activation, and release of Beclin-1 from Beclin-1–Bcl-2 complexes to initiate autophagy (77). Therefore, tubulins serve as interacting partners in the regulation of autophagy initiation.

During autophagy initiation autophagosome membranes are produced from existing intracellular membranes and microtubules are well positioned to act as carriers of these membrane components from existing organelles to sites of phagophor nucleation. Recent studies have shown that LC3 enrichment and autophagosome formation occur at contact sites between Parkin-tagged mitochondria and the ER (176). Microtubules mediate translocation of both these organelles (177, 178) and may critically regulate their co-localization to initiate autophagosome formation.

The role of microtubules in autophagosome formation is differentially regulated in basal and starvation conditions. Microtubule dynamics are required for autophagosome formation in response to nutrient starvation (77, 162) but not under basal conditions (162, 179, 180).

Once formed, autophagosomes are transported along microtubules in both anterograde and retrograde directions (77), where they are fused with lysosomes. The role of microtubules in mediating the fusion of autophagosomes with lysosomes remains controversial. Microtubule dynamics do not affect the co-localization and fusion of autophagosomes and lysosomes (162), which can occur in the absence of microtubules (160). However, Kimura et al. argue that more efficient fusion is enabled by active transport along microtubule (181). These contrary observations may be explained by the influence of pharmacological or RNA interference-based modulators on lysosomal behavior in addition to their effects on microtubule cytoskeleton. However, studies using tools that more selectively target the autophagy machinery are required to clarify the importance of microtubules in autophagosome–lysosome fusion in autophagy, and the mechanisms regulating these processes.

Overall, microtubules regulate autophagy through scaffolding functions and in the intracellular trafficking of autophagy components. While precise mechanistic details remain elusive, it is likely that tubulin alterations seen in cancer would influence autophagic function and the ability of cells to cope with microenvironmental and chemotherapeutic stressors that cause nutrient starvation and cellular damage.

PROTEIN FOLDING STRESS

Misfolded proteins may arise from protein damage, inadequate chaperone activity, and malfunction of protein processing systems. The ER is responsible for ensuring correct folding of membranous and secretory proteins and this organelle is highly sensitive to cellular conditions. Slight changes in any number of parameters can lead to accumulation of unfolded proteins in the ER lumen and initiation of the unfolded protein response (UPR) [reviewed in Ref. (182)]. The UPR involves the induction of the ER-associated degradation machinery that allows transport of unfolded proteins to cytoplasmic proteasomal systems, suppression of translation, and upregulation of chaperones in a concerted effort to reduce the burden of misfolded proteins (182). Initiation of the UPR leads to amelioration of ER stress, or the initiation of cell death (182). The UPR is upregulated in many cancers and is an important contributor to tumor development and maintenance (182–184). ER stress sensitizes cells to a broad range of chemotherapeutics including topoisomerase inhibitors (185),

temozolomide (186), platinum-based agents (187, 188), and TBAs (189).

Glucose regulated protein 78 (GRP78) is a member of the heat shock protein 70 (Hsp70) family and a master regulator of the ER stress response (190). Alterations in GRP78 expression and localization have been linked with tumor aggressiveness, migration, and invasion as well as chemoresistance, where it acts as a pro-survival factor (182). Taxanes and vinca alkaloids induce ER stress through upregulation of GRP78 in breast cancer cells (5). ER stress is also associated with JNK activation and apoptosis, which are inhibited upon GRP78 knockdown (5, 191). GRP78 interacts with βIII-tubulin (107), however, the functional consequences of this association are unknown. These observations suggest an intrinsic link between the microtubule cytoskeleton and the initiation of ER stress responses.

Tubulin-binding agent treatment also initiates mechanisms to repress translation and ameliorate misfolded protein accumulation. Treatment of cervical cancer cells with TBAs induces P-body formation, which are cytoplasmic regions where mRNA translation is inhibited (104). P-body targeting of miRNA and mRNA is also an important regulator of numerous stress responses, including the regulation of HIF1α levels in normoxic and hypoxic conditions (192). Microtubule dynamics are also critically involved in the association of mRNA with stress granules (193), which also regulate mRNA processing in response to stress (194).

Expansion of the ER network occurs during the UPR (195), where it acts to relieve ER stress (196). Microtubules are critically involved in regulating ER morphology, trafficking, and expansion of the organelle to the periphery of the cell by direct attachment of the ER to microtubules (197). Microtubule dynamics are tightly co-regulated with ER dynamics, which are suppressed by microtubule depolymerizing agents (178, 198). ER movement can occur by attachment to the microtubule plus ends (198), or kinesin-mediated ER sliding along microtubules (58, 199). While the former mechanism occurs on highly dynamic microtubules, ER sliding occurs on acetylated microtubules (58). Therefore, tubulin post-translational modifications may act as important regulators of ER expansion during the UPR. Mitochondria are also localized to acetylated microtubules, with this PTM potentially facilitating functional ER–mitochondrial interactions with diverse consequences for the cell, including autophagy induction (58, 176). Therefore, the microtubule network may co-ordinate whole cell reprograming in response to localized ER stress.

In neuronal neuroblastoma models, collapse of the microtubule network and evolution of ubiquitinated protein aggregates at the centrosome were observed in parallel with the initiation of ER stress (200). While this suggests that maintenance of a functional ER network relies heavily upon the microtubule cytoskeleton, similar observations are yet to be reported in non-neuronal cancer cells.

These observations suggest an intrinsic link between ER homeostasis, the initiation of ER stress responses and the microtubule network; however, the mechanisms co-regulating these systems remain elusive. Improved understanding of the role of microtubules in ER function, and the importance of this organelle in tumor development and cell survival may reveal strategies for more effective use of existing treatments in cancer.

TUBULIN AND MOLECULAR CHAPERONES OUTSIDE OF THE ER

Other chaperones outside of the ER system also interact with microtubules (201). The small heat shock protein (Hsp) α B-crystallin regulates microtubule dynamics (202) and tubulin polymerization (203) by associating with microtubules through interactions with MAPs (204). The association between α B-crystallin and tubulin may also prevent the aggregation of misfolded tubulin (202).

Heat shock protein 27 (Hsp27) associates with microtubules (205) and alters the microtubule structure by promoting microtubule nucleation distant to the centrosome (206). TBAs induce Hsp27 phosphorylation through the p38 signaling pathway in MCF-7 cells, with microtubule stabilizers and destabilizers inducing different phosphorylation patterns on this protein (207). However, the functional consequences of these phosphorylation sites are unclear. Hsp70 also associates with tubulin by interacting with the tubulin C-terminal tail, and this interaction may be mediated by MAP1B (208, 209). In particular, βIII-tubulin has been found to associate with mitochondria-localized Hsp70 (107). Hsp70 expression is induced by vinblastine treatment in melanoma cells (210). Furthermore, crosstalk between Hsp70 and oxidative stress enzymes (211) suggests that interactions between the microtubule network and these proteins could have profound implications for a variety of stress responses.

The Hsp90 family is the main cytosolic chaperones in basal and stressed conditions, where they mediate maturation of folded proteins (212). Hsp90 client proteins are diverse and include oncoproteins that promote survival in response to environmental stress [reviewed in Ref. (213)]. Hsp90 proteins have been found to associate with tubulin; however, this occurs in an ATP-independent manner, suggesting that tubulin–Hsp90 associations are not related to global tubulin re-folding or the targeting of tubulins to proteasome machinery (214, 215). The binding of Hsp90 to tubulins may instead ensure correct folding of nascent tubulin peptides, and prevent the formation of tubulin aggregates during cellular stress (214). The association between these proteins may also reflect the role of Hsp90 as a molecular chaperone for proteins translocating on microtubules (216).

Heat shock protein 90 recruitment to microtubules depends on acetylated tubulins, with HeLa cells having higher levels of acetylated tubulin and Hsp90 recruitment to microtubules compared with non-tumoral RPE1 cells (52). Tubulin acetylation is also associated with recruitment of the Hsp90 client proteins Akt and p53 to microtubules, with significant implications for downstream signaling events and chemosensitivity (52). Whether tubulin hyperacetylation is a widespread feature of cancers, or is specific to these cell types, is unclear, but these observations suggest that tubulin post-translational modifications may impact upon protein folding stress in cancer. Overall, interactions between tubulins and Hsp90 may act as an important link between tubulin PTMs, protein folding, and stress response signaling.

MITOCHONDRIAL FUNCTION

As integrators of cell state and mediators of apoptotic signaling, mitochondria play a critical role in determining cell fate in response to stress. There is growing evidence that tubulin, microtubules, and the microtubule network regulate

mitochondrial function in cancer (217). Microtubules are involved in mitochondrial trafficking and degradation, with these processes influencing microtubule stability and tubulin degradation (218). Tubulin is an integral component of mitochondrial membranes (136, 137, 219), and these membranes are enriched with βIII-tubulin (107, 137, 217). Mitochondria-associated βIII-tubulin is distinguished from the cytoplasmic tubulin pool by distinct post-translational modifications (107). Interactions between tubulin and VDAC discussed above, also support a role for tubulins in mitochondrial function.

Tubulin-binding agents are known to affect mitochondrial stress (115). Microtubule stabilizing and destabilizing TBAs cause changes in the mitochondrial membrane potential, which is critical for the maintenance of respiration and regulation of apoptosis (135, 220). It is currently unclear whether these effects are independent of the tubulin-targeted activity of these agents. Nevertheless, higher levels of soluble tubulin are associated with a lower mitochondrial membrane potential in cancer cells but not in non-transformed primary cells (220). Therefore, modulation of mitochondrial function by tubulin and microtubules may influence cell stress responses and cell survival signaling in cancer.

CELL DEATH SIGNALING

Failure of cellular stress responses to alleviate cellular dysfunction can result in the induction of cell death. Emerging evidence supports a role for tubulins and microtubules in the execution of cell death in response to stress. For instance, tubulins interact with regulators of mitochondrial membrane permeability and apoptosis. Interactions between tubulin, VDAC, and p53 (discussed above) may influence the mitochondrial permeability transition and regulate apoptosis induction (221). This is supported by evidence that TBAs mediate their apoptotic effects by directly compromising the mitochondrial outer membrane integrity (222), whether through interactions with their traditional target, tubulin, or with B-cell Lymphoma/Leukemia-2 (Bcl-2) (223).

Crosstalk between microtubules and apoptotic networks is also suggested by Bcl-2 involvement in TBA-mediated cell death. In leukemic cell lines, the overexpression of Bcl-2 suppresses the apoptotic response of TBAs independently of G2/M arrest and structural microtubule alterations (224–226). High Bcl-xL levels are protective against taxol-induced cell stress (225). These effects may be explained by direct interactions between Bcl-2 and tubulin (217, 227). Bcl-2 interacting mediator of cell death (Bim) is also sequestered on microtubules by binding to the dynein light chain, thereby preventing initiation of apoptotic signaling (227, 228). Once released from microtubules, Bim translocates to mitochondria, and interacts with Bcl-2, Bcl-xL, or Bax to promote apoptosis (228). Biophysical studies have also indicated that BH3-domain proteins, of which Bim is a member, can interact with tubulin through this domain (227). The pro-survival factors semaphorin 6A and survivin also associate with microtubules (107, 229, 230) with the latter affecting microtubule dynamics (229). Semaphorin 6A interacts directly with βIII-tubulin in ovarian cancer cell lines and its expression correlates with resistance to a broad range of chemotherapy agents (230). By interacting with apoptotic proteins, tubulin alterations may have a pro-survival effect by reducing the apoptotic potential of cancer cells.

Manipulation of the soluble and polymerized tubulin fractions may also modulate apoptotic potential. Bak associates with the polymerized fraction while Bid preferentially associates with the soluble fraction (227). This interaction is mediated by the β-tubulin C-terminal tail region (227), suggesting that tubulins may modulate apoptotic potential in an isotype-specific manner. However, this interaction, its tubulin isotype specificity and functional consequences are yet to be validated in the more complex cell environment.

Tubulin-binding agents are known to induce Bcl-2 phosphorylation, a state that inhibits the anti-apoptotic activity of this protein (231), suggesting that Bcl-2 activity may be regulated by microtubule integrity. However, Bcl-2 phosphorylation is elevated in cells undergoing G2/M arrest and this observation may reflect the action of TBAs on the cell cycle checkpoint, rather than apoptotic signaling (232).

Direct and indirect interactions between tubulins, apoptotic proteins, and mitochondria suggest that the microtubule network communicates with the apoptotic machinery to regulate the execution of the final stages of cell death signaling. While the precise mechanistic details of this cross-talk remain elusive, the current evidence supports a role for isotype-specific regulation of cell death by tubulins.

CONCLUSION

Tubulins, microtubules, and their interacting partners are increasingly recognized as central players in the maintenance of cell homeostasis and execution of cell stress responses. Emerging evidence suggests that the modulation of tubulin isotype composition, post-translational modifications and the expression of MAPs seen in cancer influence diverse cellular functions to promote cell survival under metabolic, protein, oxidative, and hypoxic stress. Microtubules and tubulins influence protein signaling networks through molecule and organelle transport, act as scaffolds for protein–protein interactions, modulate enzyme activity, and sequester stress response mediators. Developing a detailed spatiotemporal knowledge of the specific function of tubulin isotypes, their post translation modifications and the proteins they associate with presents a major challenge, and is a necessary foundation for understanding the role of the microtubule network in the regulation and execution of stress responses.

By influencing a variety of cell stress responses, microtubules are well positioned to act as coordinators of cell function in response to stress. Furthermore, crosstalk between different stress response signaling events means that microtubule involvement in this context may have profound implications on diverse cellular functions (**Figure 2**).

Improved understanding of the role of tubulins and microtubules in cell stress responses in cancer has appreciable clinical benefits. The identification of signaling pathways influenced by the microtubule cytoskeleton may offer a source of novel anticancer treatments. A firmer grasp on the role of the microtubule cytoskeleton in cell stress responses, and in particular in chemotherapeutic stress, should also enable more effective use of existing treatments. By profiling tubulin and microtubule aberrations in tumors, chemotherapeutic combinations known to induce

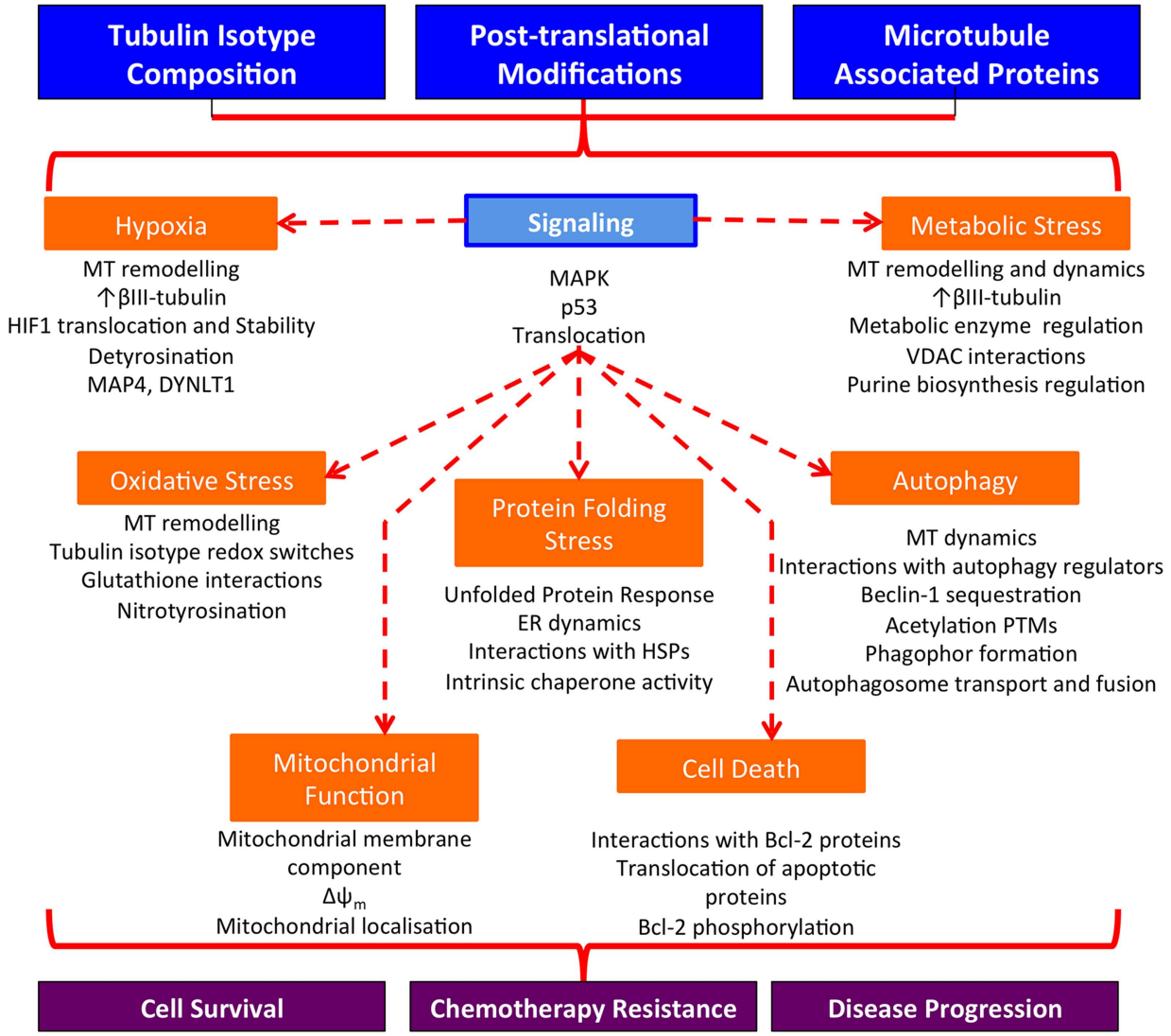

FIGURE 2 | Microtubules regulate and co-ordinate diverse cellular stress responses in cancer cells. Alterations in the expression of tubulin isotypes, tubulin post-translational modifications, and the interaction of microtubules with MAPs seen in cancer affect a wide range of homeostatic mechanisms in response to cellular stress. Microtubules may function to co-ordinate stress responses across the cell, resulting in enhanced cell survival in the harsh tumor microenvironment, resistance to chemotherapy treatment, and the development of more aggressive disease; MT, microtubules.

particular stress states could be selected to exploit altered stress response signaling in cancers. Through these avenues, a thorough understanding of the role of the microtubule cytoskeleton in stress responses has the potential to lead to larger therapeutic windows, reduced chemotherapy resistance, and more effective cancer treatment with reduced side effects.

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