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Review

Microtubule-targeted agents: When mitochondria become essential to chemotherapy ☆

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ABSTRACT

Microtubule-Targeting Agents (MTAs) constitute a class of drugs largely used for cancer treatment in adults and children. In cancer cells, they suppress microtubule dynamics, and induce cell death *via* the mitochondrial intrinsic pathway. To date, links between mitochondria and microtubule network disturbance in MTAs mechanism of action are not obvious. The aim of the present contribution is to provide elements that could answer to the question: how far are mitochondria essential to anticancer chemotherapy that targets the microtubule cytoskeleton? We review the main molecular candidates to link microtubule alteration with the apoptotic mitochondrial pathway control. Involvement of direct targeting of mitochondria in MTA efficacy is also discussed. Furthermore, we line up current evidence and emerging concepts on the participation of both mitochondria and microtubule in MTA neurotoxic side effects. To decipher the interconnections between the mitochondrial and the microtubule networks may help to improve cancer cell response to chemotherapy. This article is part of a Special Issue entitled: Bioenergetics of Cancer.

1. Introduction

Improvement of anticancer therapeutic strategies is often limited by a poor knowledge of molecular mechanisms underlying carcinogenesis and cell response to treatment. Although carcinogenesis is a very complex process, it can be divided into two crucial steps: 1) appearance of oncogene mutations in a group of cells, leading to 2) disorderly cell proliferation. This uncontrolled cell division, joined to neo-angiogenesis induction, is responsible for tumor formation, growth and spreading. As the microtubule network is highly involved in cell proliferation, it appeared to be a preferential target for cancer therapy. For that matter, great efforts have been devoted to discover drugs that affect microtubules. Nowadays, the so-called Microtubule-Targeted Agents (MTAs) constitute a class of anticancer drugs largely used in the clinics. Among them, taxanes and Vinca alkaloids are powerful inhibitors of microtubule dynamics and apoptosis inducers, used to treat solid tumors and malignant hemopathies. The therapeutic success of MTAs accounts for the development of new microtubule-targeting compounds by pharmaceutical companies, which has been – and is still – intense and fruitful.

The aim of the present contribution is to answer the question: how far are mitochondria essential to anticancer chemotherapy that targets the microtubule cytoskeleton? First, we briefly summarized the cellular effects of MTAs on microtubule dynamics, and their functional consequences. Then, as MTA anticancer effectiveness has been related to the apoptotic mitochondrial pathway, we lined up the main molecular candidates to link microtubule alteration with apoptosis control; and we discuss the direct effects of MTAs on mitochondria. We also reviewed current evidence and emerging concepts of both mitochondria and microtubule role in MTA neurotoxic side effects. Lastly, we considered MTAs as tools to study the influence of microtubule dynamics on mitochondrial dynamics.

2. MTA family of molecules, a reference in anticancer chemotherapy

Microtubules are cytoskeletal hollow filaments present in most eukaryotic cells that result from polymerization of α/β tubulin polymers. In mammalian cells, microtubules are polarized structures nucleated at the centrosome where the minus end is anchored. The plus end grows to the cell periphery and constantly explores the cytoplasm, making microtubule highly dynamic polymers. Indeed, a fundamental property of microtubules is to exhibit a dynamic instability, which is characterized by a succession of slow polymerization and rapid depolymerization phases. The switch from microtubule growth or pause to shrinkage is known as “catastrophe”, and the switch from shrinkage to growth or pause is named “rescue” [1]. This dynamic behavior is of particular importance in the regulation of many cellular functions by the microtubule cytoskeleton, as being the support for cell division, shape changes, motility and cell differentiation such as

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formation of neuronal outgrowths. Thus, far from being considered as mere architectural elements, microtubules are key determinants of cellular events and functionalities. Only a few microtubule-governed cellular processes require an overall remodeling of the cytoskeleton network, but all depend on a tightly regulated microtubule dynamics.

Microtubule-Targeted Agents (MTAs) remain benchmark clinical treatments displaying high cytotoxic efficiency and are still widely used against a broad spectrum of children's and adult's tumors. They recently received a revival of interest as potent anti-angiogenic and vascular-disrupting agents [2]. Research and development are still in progress to discover more active and less toxic compounds (for extensive reviews, see [3,4]). Attempts to improve the intracellular drug concentration and a more specific targeting of tumor cells are especially under intense investigations, and the emerging field of nanotechnologies actively participates to this quest [5]. The MTA family is composed of more than 30 compounds, historically classified in destabilizing and stabilizing agents, according to their binding site on tubulin or microtubules [3,6]. Destabilizing agents inhibit microtubule polymerization *in vitro*; they include the Vinca alkaloids such as vinblastine or vincristine that bind to the so-called "Vinca" tubulin domain, as well as nocodazole or combretastatins that bind to the "colchicine" tubulin domain. Stabilizing agents enhance tubulin polymerization and microtubule stabilization; they include taxanes, such as paclitaxel or docetaxel, and Epothilones. Although the distinction in destabilizing and stabilizing agents is useful for structure–activity studies, it is no more used in cellular and *in vivo* studies since both classes have been shown to commonly disturb microtubule dynamics, without changing the overall microtubule mass in a large range of concentrations [7].

It is now largely accepted that cytotoxic (i.e. pro-apoptotic) concentrations of MTAs suppress microtubule dynamics. [8–11]. An extensive decrease in microtubule dynamics prevents the normal alignment of chromosomes, activates the spindle assembly checkpoint, which results in the cell blockade in mitosis [12]. It should here be noticed that a moderated suppression of microtubule dynamics, which did not allow the accumulation of cells in mitosis, is also associated with apoptosis induction in tumor cells [13–15]. Thus, it remains unclear whether and how the mitotic arrest is coupled to the activation of the apoptotic machinery [16,17]. Elsewhere, microtubule dynamics suppression correlates with cell locomotion alteration, as described in fibroblasts treated with paclitaxel and nocodazole [18,19]. Surprisingly, the increase in microtubule dynamics by low concentrations of vinflunine and paclitaxel has been also shown to inhibit endothelial cell migration, resulting in MTA anti-angiogenic activities [20]. This effect was correlated with the inhibition of interphase microtubule functions, resulting in inhibition of adhesion site dynamics and formation of long-lived stress fibers [21]. Interestingly, whatever the concentration studied, MTAs disorganize the network of microtubule + end tracking proteins (+TIPs). Among them, the end binding (EB) family of proteins specifically forms comet-like accumulation at the ends of growing microtubules. As they ensure microtubule growth, EB proteins are crucial regulator of microtubule dynamics [22]. Taxanes, Vinca alkaloids and Epothilones commonly disrupt EB protein distribution in cancer, endothelial and neuronal cells [21,23] (and personal data), which may account for MTAs' main effects on interphase and mitotic microtubule dynamics.

3. Anticancer effectiveness of MTAs: mitochondria come into play

MTAs, including the newest in clinical trials, have shown a high ability to induce apoptosis [24–28]. This programmed and tightly regulated cell death was first identified in 1993, by Bhalla et al. [29], as the mechanism responsible for the anti-tumor cytotoxic effects of paclitaxel in human myeloid leukemia cells [14]. Since then, their effectiveness, even in the clinics, has been well correlated to apoptosis extent in all tumor cells. It should be noted that, even though MTAs can induce cell death in endothelial cells, it is dispensable for their anti-angiogenic and anti-vascular properties at low doses [30,31]. This section will thus be focused on apoptotic actors necessary to MTA anticancer activity in tumor themselves, giving clues to understand how can microtubule dysfunction be a necessary step in apoptosis induction.

3.1. Intrinsic pathway induction by MTAs: what to learn for onco-pharmacology?

From the numerous intracellular apoptotic signals, two major routes can be discerned: the mitochondrial pathway, known as the intrinsic pathway, and the death receptor pathway, so-called extrinsic pathway. Although MTAs have been shown to modify expression levels of death receptors and their ligands, the extrinsic pathway is generally excluded from MTA-induced apoptosis [32–34]. MTA effectiveness is largely accepted as a consequence of caspase activation through the intrinsic apoptotic pathway [35]. Of note, the anticancer activity of novel improved pharmacological features of MTAs, such as hydrophilic paclitaxel derivative or paclitaxel loaded poly(L-lactic acid) microparticles, is evaluated by measuring their impact on mitochondria [36,37]. It points out how these organelles are crucial in the appraisal of microtubule-targeted chemotherapy success.

3.1.1. The lethal cascade

Mitochondrial membrane permeabilization is the central gate in turning on/off apoptosis, as it allows the release of a large panel of pro-apoptotic proteins [38,39], that activates downstream signaling cascades and leads to the final execution of cell death. An early and transient hyperpolarization of the mitochondrion has been reported with MTAs, which is, in tumor cells, followed by the $\Delta\Psi m$ collapse and the release of pro-apoptotic factors [17,30]. The subsequent activation of the caspase cascade is the non-return point to cell biochemical destruction and phenotypic changes in MTA-induced apoptosis [35,40–43]. In response to paclitaxel- or epothilone-treatment, the massive cytochrome *c* release from mitochondria triggers the formation of the multi-factor complex apoptosome, which leads to an early increase in caspase-9 activity. Accordingly, the caspase-9 specific inhibitor (z-LEHD-fmk) effectively protects cells from MTA-mediated apoptosis [42–44]. Overexpression of Apaf-1, the adaptor molecule of the apoptosome, has been shown to enhance paclitaxel-induced apoptosis [45]. In situations where activation of caspase-9 is disturbed, overexpression of the downstream effector caspase-3 restores sensitivity to MTAs in resistant cancer cells [46]. Smac/Diablo and Omi/Htra2 peptides are also released from mitochondria during their permeabilization, and favor caspase activity by preventing action of the inhibitor of apoptosis proteins (IAPs) [39,47]. Inhibition of IAPs can, in vitro, modulate the efficacy of antineoplastic agents. Smac/DIABLO peptide enhanced the induction of apoptosis and long term antiproliferative effects of paclitaxel in breast cancer cells [48,49]. Combination of paclitaxel with a recombinant adenovirus encoding Smac/DIABLO also produced greater levels of apoptosis in ovarian carcinoma cells [50]. In addition, ectopic Smac/DIABLO sensitized drug-resistant epithelial ovarian cancer cells to paclitaxel-induced apoptosis [51]. Thus, an increase in Smac/DIABLO activity seems to be a promising strategy to improve MTA treatment, including in drug-resistant cancer, but a clinical approach is still lacking.

3.1.2. Bcl-2 family members

Much effort has been directed toward elucidating the mechanism of mitochondrial membrane permeabilization, and, while still discussed, it is now largely accepted that this process is under the control of the Bcl-2 family members. The Bcl-2 family is composed of up to 30 proteins that can be divided into 3 groups: one of Bcl-2-like survival factors and two others of cell death promoting factors named Bax-like and BH3-only [52]. The relative levels of anti- and pro-apoptotic clans in mitochondrial

membranes arbitrate cell life or death decision. MTAs modulate both expression levels and activity of pro-apoptotic members of the Bcl-2 family. Up-regulation of the Bad, PUMA, Bax and/or Bak has been observed after treatment with paclitaxel, epothilone B as well as with *Vinca* alkaloids [15,53–55]. As expected, Bcl-X_S, Bax or Bad overexpression sensitizes cancer cells to paclitaxel and vincristine [56–58]. In addition, MTAs trigger Bax activation through its conformational change [54,59] that allows N-terminal domain exhibition and Bax stable insertion into the outer mitochondrial membrane [60,61]. MTAs also initiate Bim translocation from microtubules to mitochondria, as discussed in the next section. Lastly, the late cleavage of Bid into a functional fragment (tBid) has been proposed to be a signal amplification loop which could be required for an optimal release of mitochondrial factors following MTA treatment [62,63].

In parallel, Bcl-2-like anti-apoptotic proteins can be post-translationally inactivated by hyperphosphorylation induced by a large panel of MTAs [15,64–68]. It has initially been thought to be a marker of mitosis rather than an apoptosis-related signal, but both the extent and the duration of the mitosis-associated Bcl-2 hyperphosphorylation is likely to distinguish a pre-apoptotic cell from one destined to divide [69]. Treatments with MTAs also decrease expression levels of Bcl-X_L and Bcl-2 to activate the intrinsic pathway [70–72]. Overexpression of the anti-apoptotic proteins Bcl-2 and Bcl-X_L are involved in resistance of cancer cells to microtubule-targeted chemotherapy [73–76]. Thus, Bcl-2 and/or Bcl-X_L antisense strategies have been developed, and were first reported to mediate an increase in docetaxel- and paclitaxel-sensitivity *in vitro* and in mice xenografts [33,77–79]. ABT-737, a BH3-mimetic that antagonizes Bcl-2, Bcl-X_L, and Bcl-w, increased MTA pro-apoptotic effects in a variety of tumor cell lines, including breast cancer cells with acquired resistance to paclitaxel [80–82]. Similarly, A-385358, a small molecule with relative selectivity for binding to Bcl-X_L potentiated the activity of paclitaxel in non-small-cell lung cancer cells, *in vitro* and *in vivo* [83]. In 2003, a phase II clinical trial of oblimersen (antisense oligonucleotides targeting Bcl-2) in combination with docetaxel validated progression into phase III for patients with advanced hormone refractory prostate cancer. However, Bcl-2 inhibition did not always succeed in enhancing treatment effectiveness [84–87]. Furthermore, it should be used with caution in combination with MTAs since works showed that Bcl-2 down-regulation is responsible for an unexpected resistance to paclitaxel and vinflunine in ovarian cancer cells [17,88]. Accordingly, Bcl-2 overexpression can increase non-small cell lung cancer sensitivity to docetaxel [89]. Similarly, prostate cancer cells can adapt to antisense RNA targeting Bcl-xL, leading to a paradoxal resistance to docetaxel and vinblatine [90]. This dual role of "prosurvival" mitochondrial proteins points out the need for further investigation to elucidate their real contribution in MTA treatment effectiveness and their potential as target for clinical antisense strategies.

3.2. Proteins released from the microtubule to mitochondria: at the doorsteps of MTA effectiveness?

While the main effects of MTAs on both the intrinsic apoptotic signaling cascade and the microtubule network are now quite well understood, clear links are still lacking between the two events. Since apoptosis and proliferation are closely related [91], effects of MTAs on proteins that control both phenomena have been studied for years. Cell cycle is a tightly regulated process, and its disturbance by MTAs may participate in the mitochondrial apoptotic pathway initiation. Therefore, studies on how cell cycle checkpoints modulate the intrinsic pathway are of major interest, and extensively reviewed [27]. The present section will be focused on protein candidates that could build molecular bridges between microtubules and the apoptotic machinery. Indeed, microtubules serve as scaffolds for different signaling molecules, extending the list of biological processes regulated by the microtubule network in cells. Among microtubule-linked components, regulators of the apoptotic process such as p53 and Bim may be released from microtubules towards mitochondria. Since polymerizing and depolymerizing MTAs display the common property of suppressing microtubule dynamics, it probably explains why the involvement of the microtubule-transported factors in apoptosis is similar amongst these anticancer drugs.

3.2.1. p53, the multifaceted molecule

Different lines of evidence indicate that p53 up-regulation and activation are required for maximal cell sensitivity to Taxanes, Vinca alkaloids and Epothilones [15,54,71,92–96]. The role of p53 in apoptosis mediated by microtubule disturbance is reinforced by recent data showing that overexpression of the microtubule-associated protein Tau rendered neuroblastoma cells resistant to apoptosis by mechanisms involving reduction of p53 level [97]. MTA concentration seems to be critical, as low doses of paclitaxel increase p53 protein levels, whereas high doses do not affect or even inhibit these levels [72,98,99]. Similarly, low doses of vinflunine up-regulate p53, while high doses increase p53 to a lower extent [17]. The concentration-dependent activation of this transcription factor may result from its microtubule-governed transport. Indeed, its dynein-dependent transport to the nucleus is thought to be a consequence of microtubule dynamics suppression by both stabilizing and depolymerizing agents [92,95,100,101]. High concentrations of MTAs probably induce too extensive damages to microtubules, which can no more serve as tracks for p53 trafficking. Once in the nucleus, p53 transcriptional properties are activated, leading to modulation in gene targets such as p53 itself or p21 and, more interestingly, members of the Bcl-2 family [36,102,103]. Under MTA treatment, p53 induction has been shown to down-regulate Bcl-2 and up-regulate Bax [15,71,104,105]. Recently, we identified a novel binding site of p53 on the Bcl-2 promoter, responsible for the transcriptional down-regulation of Bcl-2 by vinorelbine in breast carcinoma cells [104]. The BH3-only members PUMA and Noxa can also be up-regulated following p53 induction [54,106,107], but the role of MTA-mediated disturbance of microtubule dynamics in this process remains to be characterized.

Besides its ability to modulate the pro-apoptotic/anti-apoptotic ratio in favor of apoptosis, p53 induces apoptosis through a transcription-independent mechanism. In response to various pro-apoptotic stimuli, including MTAs, p53 rapidly moves to the mitochondria in cellular and mice models [15,93,108–111]. Once at the mitochondrion, p53 primarily associated with the outer mitochondrial membrane, but a small subfraction may be located within the mitochondrial matrix [108,111–113]. The mitochondrial p53 participates in the apoptotic cascade by inducing the mitochondrial outer membrane permeabilization through direct activation of Bax-like and BH3-only proteins, and by forming inhibitory complexes with Bcl-2-like members [106,114]. The p53-targeted drug pifithrin-μ, which blocks the interaction of p53 with Bcl-X_L [115], inhibits a part of vinorelbine-induced apoptosis [104], supporting a role for the mitochondrial p53 in MTA pro-apoptotic properties. While some key steps have been invoked to explain how p53 translocation from microtubules can be triggered [74,115], the exact role of MTA-induced damages of microtubule dynamics is still an unexplored field.

3.2.2. Bim goes for a walk to mitochondria

BH3-only member of the Bcl-2 family, Bim is involved in inhibition of metastasis formation and in the pro-apoptotic response of tumor cells to chemotherapy [116–121]. Bim expression level increases dramatically after paclitaxel treatment [99,122] and gene silencing experiments showed that its up-regulation is a critical regulator of apoptosis in cancer cells [122–124]. While Bim can be localized at mitochondria without cell death stimuli in some cellular models, number of studies showed that it is sequestered by microtubules *via* its interaction with the dynein light chain DLC1/LC8 of the motor complex or *via* a direct interaction with microtubules [125,126]. Interestingly, Bim-deficient lymphocytes are less sensitive to paclitaxel-mediated perturbations of

the microtubule network, which underscores the importance of Bim in mediating apoptosis induced by agents that target microtubules [127]. During treatment with paclitaxel, Bim may act as a sensor of cytoskeleton integrity, since it is unleashed from microtubules and translocates to mitochondria [123]. In support to this hypothesis, the plant toxin called persin has been shown to induce Bim release by acting as a microtubule-stabilizing agent in breast cancer cells [128]. Similarly, the HIV-1 Tat protein activated apoptosis in host cells by triggering Bim translocation to mitochondria in response to microtubule stabilization [127]. We also showed that epothilone B induced Bim accumulation to mitochondria, leading to apoptosis in human neuroblastoma cells [93]. Since then, works have confirmed that MTAs freed Bim from microtubules and enriched mitochondria in this pro-apoptotic protein [129,130].

Bim sequestration from dynein may be released through its phosphorylation by JNK, as described with UV treatment [116,131]. Such a kinase can be activated by MTAs [27]. However, it may also be argued that Bim translocation triggered by MTAs results from disturbance of microtubule dynamics or structure. Indeed, microtubule destabilization by Gadd45a led to Bim release without activation of JNK [132]. Among the different hypotheses, the enhanced generation of mitochondrial reactive oxygen species (ROS) by MTAs [35,41,133–136] has been shown to be responsible for Bim accumulation to mitochondria in neuroblastoma cells [93]. Thus, the mitochondrial compartment itself can initiate the signaling dialog with the microtubule network, which results in apoptosis and thus participates in MTA efficacy.

4. Direct effects of MTAs on mitochondria: where do we stand?

In parallel with their effects on the microtubule network, MTAs can also activate the apoptotic pathway through a direct action on mitochondria. Incubation of mitochondria isolated from tumor cells with either Taxanes, Vinca alkaloids or Epothilones provokes cytochrome *c* release [137]. In contrast, other classes of anticancer drugs such as 5FU or doxorubicin were not able to permeabilize membranes from isolated mitochondria [138] (and personal data). MTAs induce an early $\Delta\Psi m$ collapse and a subsequent large amplitude swelling of isolated mitochondria [88,136,138,139]. The mitochondrial membrane permeabilization is inhibitable by cyclosporine [138], consistently with the permeability pore-dependent Ca^{2+} loss from isolated mitochondria induced by paclitaxel and nocodazole [139,140]. Paclitaxel has also been shown to significantly increase the cytochrome oxidase-mediated ROS production by purified mitochondria [136]. Interestingly, the pro-apoptotic effects of paclitaxel can be enhanced by improving its specific delivery to mitochondria using a mitochondria-specific nanocarrier system (DQAsomes) [141].

Then, it raises the question of the potential target(s) of MTAs in mitochondria. Tubulin, that was found to be strongly associated with mitochondrial membranes in both purified organelles and whole cells [139,142,143], was the first candidate proposed to explain the specific effect of MTAs on isolated mitochondria. The mitochondrial tubulin subfraction is enriched in class III β-tubulin (TUBB3), but, in contrast with the cytoskeletal form, its overexpression does not correlate with cell resistance to MTAs [144]. An association has been reported between the mitochondrial tubulin and VDAC [142,145], the major outer membrane pore that is likely involved in the release of pro-apoptotic factors by MTAs from the intermembrane space to cytosol. The current knowledge on the MTA-induced intrinsic pathway has rejuvenated the study from Evtodienko et al. [146], which suggested the involvement of either mitochondria-bound tubulin *per se* and/or contacts between mitochondria and microtubules in regulation of mitochondrial membrane permeability [146]. More than 10 years later, the C-terminal tail of tubulin has been proposed to modulate the VDAC opening and the mitochondrial respiration rate [145].

Bcl-2 has also been identified as a potential target for paclitaxel by phage display and a chemical approach [147,148]. Recently, Ferlini et al. revealed that, in ovarian cancer cells, paclitaxel directly targeted Bcl-2 in the loop domain [149]. As a result, paclitaxel changed the role of Bcl-2 from inhibitor to enhancer of the mitochondrial membrane permeabilization, facilitating apoptosis. This process may explain why the down-regulation of Bcl-2 is responsible for an unexpected resistance to MTAs in different tumor cell types and cancer patients [17,66,88,89]. Finally, the two hypotheses have joined when the association between the tubulin and Bcl-2 has been revealed, by co-immunoprecipitation from mitochondrial lysates and with purified proteins [17,88,150]. Such a complex that gathers tubulin, Bcl-2 and VDAC could be both a direct target for MTAs and a regulator of the mitochondrial membrane permeability. Thus, while the mitochondrial targets of MTAs are probably not all defined, it is reasonable to think that these anti-tumor agents display crucial anti-mitochondrial properties involved in their efficacy.

The relevance of this phenomenon remains difficult to prove in whole cells, since the direct effects of MTAs on the mitochondrial network are usually undistinguishable from those resulting from microtubule modifications. Moreover, results showing that interference of paclitaxel with the mitochondrial signaling cascade occurred upstream of microtubule organization alteration [140] should be reevaluated by measuring microtubule dynamics, a highly more sensitive parameter than microtubule architecture. Nevertheless, we showed that the early production of ROS from mitochondria was necessary to Bim translocation towards mitochondria, which in turn triggered apoptosis in human neuroblastoma cells [93]. These data strongly suggest that some of the most rapid effects of MTAs on the intrinsic apoptotic pathway may be initiated through a direct action on mitochondrial integrity. The involvement of MTA-mediated ROS generation from mitochondria needs to be reconsidered in processes such as EB protein comet disruption by MTAs (see section 2), which is thought to result from targeting of the microtubule system, but which has been recently shown to be triggered by H_2O_2 [151]. Then, it is easy to speculate that some alterations of the microtubule dynamics induced by MTAs could be, at least in part, linked to the drugs' anti-mitochondrial properties.

5. Mitochondria–microtubule pair: MTAs stir up the trouble in neuronal system

Chemotherapy-induced peripheral neuropathy (CIPN) is the main dose limiting side of a large panel of MTAs [152,153]. Most of the time, peripheral neuropathy reverses if the treatment is stopped. However, in some cases, recovery from symptoms is incomplete and a long period of regeneration is required to restore function [154]. This neurotoxic side effect is still an unsolved clinical issue, so the ways that cytoskeleton and organelles interplay and how MTAs alter these relationships in neuronal models are of high importance.

5.1. Animal models of chemotherapy-induced neuropathy: slipping from microtubule to mitochondrial involvement

Despite intensive efforts in the development of neuroprotective agents (recently reviewed in [155]), to date, there are no approved therapies for prevention or treatment of neuropathies triggered by MTA chemotherapy [152,153,156]. This is partly due to the poor understanding of mechanisms underlying MTA-induced neurotoxicity, and thus to a lack of a valuable method of standardization in the clinical measurement of CIPN. As a postulate, it has often been declared that MTAs similarly affect the microtubule network in cancer and neuronal cells, but evidence was not always sustained. For 20 years now, animal models of CIPN have been developed (listed in [157]), attempting to investigate MTAs mechanism of action on the peripheral nervous system. Most of these models are essentially

focused on the report of pain-related behavior and only a few go on further on neurophysiological experiments. While rat models of vincristine-induced peripheral neuropathy described abnormal microtubule assemblies and densities as main damages [158,159], these microtubule profiles were not observed with paclitaxel in rat models [160]. It has thus been hypothesized that the microtubule network was likely not the only target of MTAs to be involved in neuron dysfunctions. In parallel, the deciphering of MTA action progressively slipped considerations from microtubules to mitochondria. *In vitro* data using paclitaxel reported anterograde and retrograde axonal transport blockade in rat dorsal root ganglia (DRG) and hippocampal neurons. Then, *in vivo* studies showed a significant increase in the incidence of swollen mitochondria in axons after paclitaxel treatment [160,161]. Similar data were obtained with *in vitro* culture of DRG, in which the induction of atypical mitochondria by paclitaxel was associated with a significant reduction of their functioning and the loss of mitochondrial membrane potential [162]. Nevertheless, in these studies, the link between microtubule density and inhibition of neuronal organelle distribution remained controversial due to variable paclitaxel injections modes and different administered concentrations over the time [163,164].

5.2. Inhibition of MTA's neurotoxic effects: do mitochondria need to be protected?

Many works are attempting to find clinically efficient neuroprotectors able to enhance neuronal cell survival. To date, several neuroprotective agents like thiols, neurotrophic factors and antioxidants have been tested in preclinical models and clinical trials for their ability to prevent CIPN [165,166]. Although several of these compounds were identified as neuroprotective molecules of interest, clinical data are still discussed. Mitochondrial dysfunction and oxidative stress are widely believed to underlie the pathogenesis of various neurodegenerative diseases [167]. The investigation of neuroprotective antioxidants was thus rationalized as a promising strategy to prevent or alleviate mitochondrial damages. Among them, efficacy of acetyl-L carnitine and more recently alpha lipoic acid to exert neuroprotective effects against MTAs, *in vitro*, was associated with a reduced incidence of swollen and vacuolated mitochondria in rat C-fiber [161] as well as in sensory DRG neurons [162]. Moreover, alpha lipoic acid prevented the early loss of membrane potential differential in mitochondria exposed to paclitaxel, thus preventing neurons from mitochondrial energetic failure probably through antioxidant activities. An attractive strategy, the mitochondrial protection might be limited in preventing MTA-mediated CIPN which involves alteration of other targets such as the microtubule cytoskeleton. In this context, olesoxime (TRO19622) appeared as a promising drug candidate to treat the neurotoxic side effects of microtubule-targeted chemotherapy [168]. This new molecule protected neuronal cells from MTA-induced neurite shrinkage by restoring both microtubule dynamics – through EB protein comets maintaining at microtubule ends – and the microtubule-governed mitochondrial trafficking [23]. Thus, compounds like olesoxime that are able to join these two properties would hold promise to better prevent and cure patients suffering from neurodegenerative disorders in which microtubule-associated axonal transport is defective. Their study may also bring additional fundamental insights into the molecular mechanisms underlying neurotoxic properties of MTAs.

6. Influence of microtubule dynamics perturbation on mitochondrial dynamics: a new field of investigation?

Shortly after their successful use in the clinics, MTAs have been extensively employed in fundamental research. By modulating microtubule architecture and dynamics, they are appropriate pharmacological tools to probe the mitochondria–cytoskeleton interactions.

6.1. How can MTAs modulate the mitochondrial motility?

MTA effects have been especially studied in neuronal cells, in which mitochondria move throughout the neuronal processes to contribute to synaptic maintenance. Appropriate positioning of the mitochondrial network ensures organelle function and is necessary to cell survival and functionality. As soon as 1978, Chan KY and Bunt AH used vinblastine to form paracrystal structures and to highlight the interconnected spatial organization of microtubules and mitochondria in synaptosomes and axon terminals of rat cerebral cortex [169]. A few years later, axonal organelle transport has been shown not to be totally suppressed after microtubule disruption [170], and to be partially inhibited by the introduction of agents that specifically disrupt actin microfilaments [171–173]. In parallel, the discovery and characterization of microtubule-based motors kinesin and dynein allowed to better envisage the axonal transport system. Complementary data, intending to decipher the importance of microtubule-governed transport among other cytoskeleta, used a model of neurons grown with vinblastine. Results showed that the whole mitochondrial compartment concentrated into the cell body, suggesting that microtubules were necessary and sufficient for the transport of mitochondria in axons [174].

Considering the uncontested role of microtubules as tracks for the intracellular trafficking of mitochondria, it can be argued that mitochondrial transport defects could result from microtubule dynamics alteration. In support to this, the parkinsonian toxin (MPP+) has been recently shown to induce an early alteration of microtubule dynamics and orientation, and a subsequent mitochondrial transport impairment [175]. Works in tumor cells showed that paclitaxel increased the speed of mitochondrial movement, whereas colchicine and nocodazole retarded it [123,176], suggesting that microtubule stabilization could be necessary to organelle trafficking. Up to now, the major hypothesis explored was the changes in molecular motor binding to the microtubule railways, through tubulin post-translational modifications (PTMs). Indeed, binding of the motor protein kinesin-1, that mostly ensures anterograde mitochondrial transport in axons, is increased by microtubule detyrosination and acetylation [177,178]. These two major PTMs correlate with microtubule stabilization and are induced by paclitaxel and ixabepilone in cancer cells [179,180]. These data were supported by observations of paclitaxel inhibitory effects on fast retrograde transport in rat peripheral nerves [163,181]. However, results are still controversial since a recent work showed that paclitaxel abolished kinesin-1 translocation in polarized neurons by increasing the overall levels of tubulin acetylation, detyrosination and polyglutamylation [182]. One explanation could be that, while microtubule stabilization is necessary for the mitochondrial transport, its over-stabilization compromises the intracellular trafficking.

Elsewhere, microtubule associated protein (MAPs) binding to microtubules can also influence motor-based axonal transport, mainly by affecting the attachment and detachment cycle of the motors. In neurons, tau and MAP4 can control the intracellular trafficking by reducing the attachment of kinesin to microtubules [183,184]. More recently Seitz et al. showed a decrease in run-length for both kinesin or dynein when MAP2c and tau were overexpressed in cells, combined with a significant decrease in kinesin attachment frequency on taxol-stabilized microtubules [185]. Then, as paclitaxel has been shown to increase MAP2 affinity for microtubule [186], it could thus easily be thought that paclitaxel by regulating MAPs binding could modulate organelle trafficking.

Lastly, the $p150^{Glued}$ subunit of dynactin is a +TIP (cf part 2) that, in association with dynein, participates to organelle retrograde transport. Interestingly, $p150^{Glued}$ interaction with EB1 at microtubule plus-ends seems to be central in the dynein/dynactin function [187]. Thus, by significantly disturbing EB1 localization [21,23], MTAs may cause the loss of both microtubule dynamics and mitochondrial transport, which together might lead to cancer cell death. Same observations could be

transposed to the neuronal model as EB family members are crucial for neurite growth and maintenance, and are tools of choice to precisely measure plus-end microtubule dynamics by live microscopy [188]. Interestingly, neurotoxic concentrations of paclitaxel have been shown to induce a decrease in the number and length of EB3 comet tails in Aplysia neurons [189]. Moreover, paclitaxel significantly disturbed the microtubule polar orientation, by reducing the percentage of microtubules with plus ends facing the axon tip and increasing those with plus ends facing the cell body [189]. All these microtubule modifications were associated with a severely impaired mitochondrial transport. In agreement with these data, we showed that paclitaxel and vincristine suppressed EB1 and EB3 accumulation at microtubule plus-ends, and significantly reduced the mitochondrial motility in human differentiated neuronal cells [23].

6.2. Can MTAs disrupt the fission/fusion equilibrium?

Recently, with the emergence of the neuropathology field of research, studies have flourished suggesting that mitochondrial dysfunctions are early and causal events in many neurodegenerative diseases [190] such as amyotrophic lateral sclerosis, Alzheimer's, Huntington's or Parkinson's diseases. One potential cause of mitochondrial dysfunction is the disruption of the highly controlled equilibrium between mitochondrial fission and fusion. Excessive fission or defects in fusion alter cell functions and viability through impairment of mitochondrial motility, decrease energy production, and increase of the oxidative stress [191]. Examples are given with studies using taxol at concentrations responsible for microtubule strong stabilization and leading to disruption of mitochondrial fission/fusion balance [192] as well as their ability to fastly move and distribute towards high energy demand subcellular locations [193]. In these studies, MTAs have been employed at high concentrations during very short time of exposure (less than 24 h) to induce microtubule modifications and thus to analyze mitochondrial dynamicity parameters. There is now a crucial need in reconsidering the concentrations employed. Indeed, lower concentrations may give complementary cues to untangle mitochondria and microtubule interconnections and may help to decipher how the anti-microtubule properties of MTAs lead to disturbances in mitochondrial dynamics. In tumor cells, such moderated concentrations of MTAs significantly alter microtubule dynamics and induce the mitochondrial network fragmentation, as an early process associated with their pro-apoptotic, anti-angiogenic and neurotoxic activities [23]. As previously shown with BH3-only peptides [194], our recent works suggested that this process could result from Bim accumulation in mitochondrial membranes (Savry et al, submitted), by a molecular mechanism that should be investigated.

7. Conclusion

To conclude, it clearly appears that MTAs are both anticancer drugs with a high clinical value and very useful tools to analyze the roles played by the microtubule network in physio/pathological processes. To decipher the tangle of MTA-induced apoptotic signals is a tricky exercise and, to date, it is still difficult to determine whether biochemical events that lead to apoptosis are activated downstream or upstream inhibition of microtubule dynamics and functions. However, it becomes clear that crucial molecular links are established between the microtubule network and the apoptotic machinery, to ensure the success of the cell death program. In that sense, analysis of mechanisms responsible for tumor cell resistance to MTAs would also provide key information about the close connections between microtubules and the apoptotic machinery. The coexistence of modifications in the microtubule system and the mitochondrial signaling cascade in cells resistant to MTAs [17,179,195] strengthens the need for novel insights into interconnections between the two compartments to help circumventing this clinical problem. It also confirmed that the mitochondrion is still a promising therapeutic target that could improve combinatorial therapy with MTAs and provide crucial arms to help treating cancers.

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