Institute of Pharmaceutical Biology[1], Biocenter, Goethe-University, Frankfurt am Main; Pharmaceutical Biology[2], Department of Pharmacy – Center for Drug Research, University of Munich, Germany

A new perspective on old drugs: non-mitotic actions of tubulin-binding drugs play a major role in cancer treatment

R. Fürst[1], A. M. Vollmar[2]

Received November 7, 2012, accepted November 21, 2012

Prof. Dr. Robert Fürst, Institute of Pharmaceutical Biology, Biocenter, Goethe-University, Max-von-Laue-Str. 9, 60438 Frankfurt, Germany
fuerst@em.uni-frankfurt.de

Dedicated to Prof. Dr. Theo Dingermann, Frankfurt, on the occasion of his 65th birthday.

Pharmazie 68: 478–483 (2013) *doi: 10.1691/ph.2013.6509*

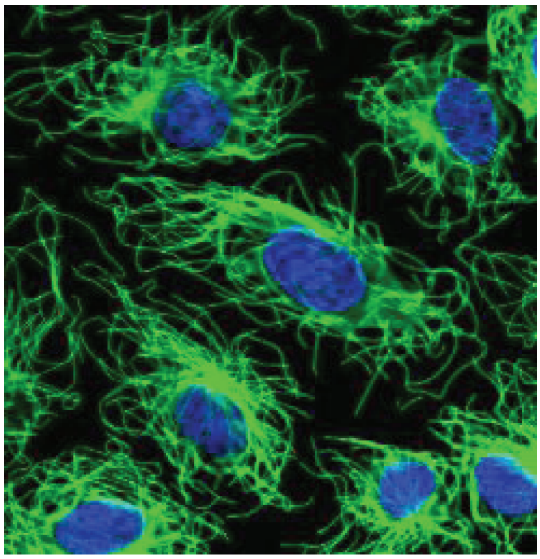
Microtubule-targeting agents (MTAs) are the most frequently used anti-cancer drugs. They can be divided into tubulin stabilizing and destabilizing agents. Their mode of action has been ascribed to their ability to interfere with the spindle apparatus and, thus, to block mitosis leading to tumor cell death. However, this view has been challenged in the last years and it became increasingly evident that non-mitotic actions of MTAs, *i.e.* their ability to affect the dynamics of interphase microtubules, are the most relevant mechanism underlying their efficacy. In this review we are presenting a distinct selection of examples of studies describing biological effects of MTAs in three areas: (i) mitosis-independent cell death and metastasis, (ii) tumor angiogenesis, and (iii) vascular-disrupting activity.

1. Microtubules

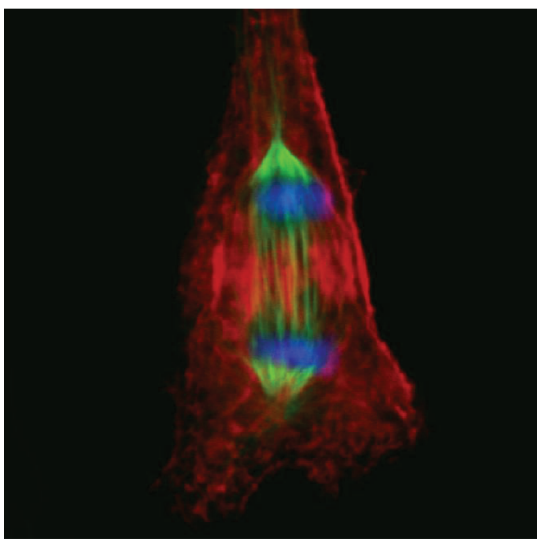
Besides microfilaments (actin) and intermediate filaments, microtubules are a pivotal component of the cell's cytoskeleton and are of high importance for many cellular events including cell motility, maintenance of the shape of a cell, organelle distribution, intracellular transport and signaling processes, and—due to their pivotal function in the spindle apparatus—for chromosome segregation during mitosis. They represent fibrillar polymeric structures that are made up by the spherical monomers α- and β-tubulin (each about 50 kDa). Several isoforms of α- and β-tubulin exist and they exhibit cell type-specific patterns of expression as well as a large number of post-translational modifications, such as phosphorylation, acetylation, or palmitoylation. The lateral assembly of tubulin heterodimers forms protofilaments that assemble to long pipe-like structures with a diameter of approximately 25 nm. Microtubules are polarized: At the (+)end only β subunits and at the (-)end only α subunits are exposed. The (-)end is anchored at the microtubule-organizing center (MTOC) of the cell (also called centrosome in the interphase), which is mainly composed of a third form of tubulin, γ-tubulin, and located near the nucleus and the Golgi apparatus. The (+)end goes into the periphery of the cell and is highly dynamic, meaning that both assembly and disassembly events occur at this region leading to growth (called "rescue") or shrinkage (called "catastrophe") of the microtubule ("dynamic instability"). Moreover, GTP hydrolysis is strongly involved in these dynamic processes as energy providing mechanism. Figure 1 shows the typical distribution of interphase tubulin in a primary human (endothelial) cell and the arrangement of tubulin during mitosis (spindle apparatus).

2. Prominent microtubule-targeting agents (MTAs)

Due to the involvement of microtubules in the spindle apparatus and, thus, cell division microtubule-targeting agents (MTAs) have been exploited as highly valuable drugs that exert cytotoxic effects on tumor cells, *i.e.* mitotic arrest followed by cell death. In fact, MTAs are the most frequently used class of anti-cancer drugs and have been introduced into the clinic in the early 1960 s. They can easily be divided into two categories according to their action: microtubule stabilizers and destabilizers. Without exception, all therapeutically applied MTAs represent natural products or their semisynthetic derivatives. Their chemical structures are depicted in Fig. 2. Destabilizing drugs that are currently used are the *Vinca* alkaloids (extracted from the plant *Catharanthus roseus*) vincristine, vinblastine, vinorelbine, vindesine, and vinflunine. They are used against hematological and lymphatic neoplasms and also against a number of solid tumors (Yue et al. 2010). Also colchicine (from *Colchicum autumnale*) belongs to the group of destabilizing agents, however, it is not used for anti-cancer treatment, but has recently been approved by the FDA for the therapy of the inflammatory diseases familial Mediterranean fever and gout. Tubulin-stabilizing anti-tumor drugs are the taxans paclitaxel and docetaxel, which are originally gained from bark of *Taxus brevifolia*, as well as the newest MTA, the epothilone B analog ixabepilone, which is a product of the myxobacterium *Sorangium cellulosum*. The taxanes are used for the treatment of solid cancers, such as lung, breast, prostate or ovarian tumors, and ixabepilone has recently been approved by the FDA for the therapy of advanced breast cancer that no longer reacts on established chemotherapeutics. Three major ("classic") binding sites of the aforementioned agents have been characterized on β-tubulin: the *Vinca* alkaloid-, the colchicine-, and the taxane-binding site. A great variety of experimental MTAs currently undergoes preclinical and/or clinical testing. Prominent compounds are combretastatin A4, hemiasterlins, laulimalides, pelurosides, halichondrins, tubulysins, pretubulysin, spongistatin, dolastatins, and discodermolide.



confluent endothelial cells during interphase



single endothelial cell during mitosis

Fig. 1: Tubulin in human endothelial cells – distribution of tubulin in the interphase and during mitosis. Endothelial cells were stained with an anti-alpha-tubulin antibody (green). Hoechst 33342 (blue) was used to visualize the nucleus. Red staining represents F-actin.

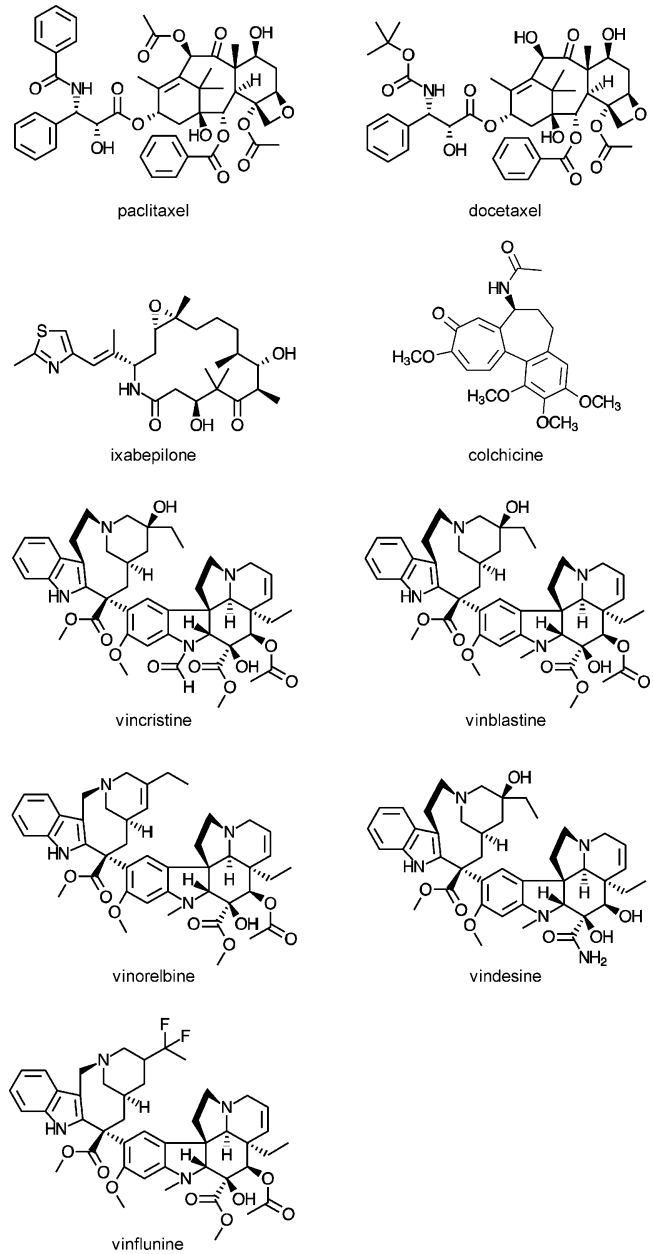


Fig. 2: Chemical structure of therapeutically used MTAs.

3. The anti-cancer effects of MTAs are based on their non-mitotic actions

The anti-cancer effects of tubulin-targeting drugs have frequently been associated with the inhibition of mitosis. However, an interesting perspective has recently been discussed arguing that mitosis is not a key target of MTAs in patients: There is a growing body of evidence that the non-mitotic actions of these drugs by interfering with interphase processes are eventually the pivotal underlying mechanisms for their clear-cut therapeutic efficacy (Komlodi-Pasztor et al. 2011). One major reason for the overestimation of the relevance of mitosis inhibition is the fact that MTAs have primarily been tested in cell culture and xenograft models, where cells divide and grow much more rapidly than the most tumor cells in patients do (Komlodi-Pasztor et al. 2011). Interestingly, addressing mitosis-specific drug targets, such as the polo-like kinases, the aurora kinases, or the kinesin spindle protein, has therapeutically not been overly successful up to now. Moreover, this approach was thought to eliminate the major side effects of MTAs, neurotoxicity, which is mitosis-independent, but came up with neutropenia as dose-limiting cytotoxic action (Komlodi-Pasztor et al. 2011).

In the last years it became increasingly evident that targeting the tubulin cytoskeleton by MTAs influences crucial cellular functions beyond affecting the mitotic spindle apparatus: MTAs (i) induce mitosis-independent cancer cell death and inhibit metastasis, (ii) inhibit tumor angiogenesis, and (iii) act as vascular-disrupting agents. In concern of these important new facts, this mini review intends to widen up our view regarding the mode of action of these compounds. A distinct selection of examples of work describing biological effects of MTAs in the mentioned three areas (overview in Fig. 3) will highlight the potential and importance of established and experimental MTAs.

4. Mitosis-independent induction of cell death

The intracellular apoptotic cell death program is strongly regulated by modulation of activity of members of the Bcl-2 (B-cell lymphoma/leukemia-2) and IAP (inhibitor of apoptosis) family of proteins (Fulda et al. 2012; Kelly et al. 2011; Ola et al. 2011;

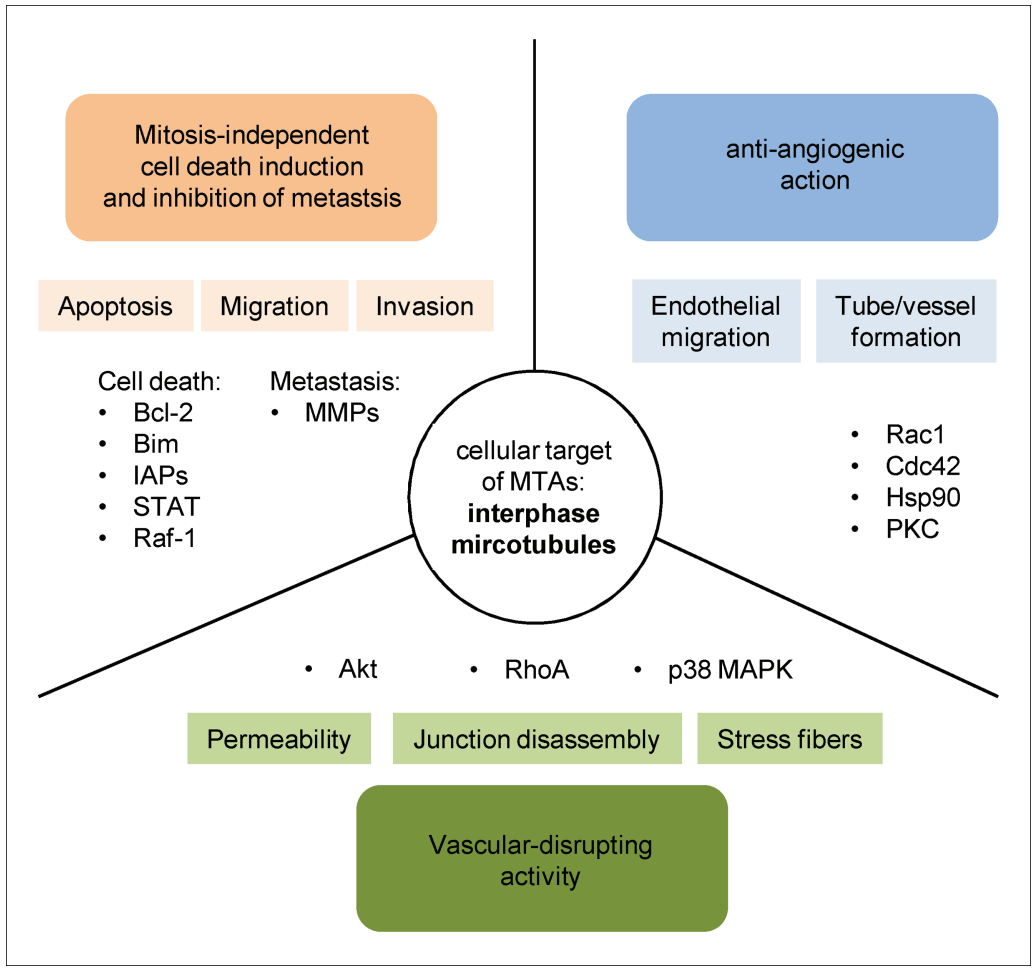


Fig. 3: Summary of the mitosis-independent cellular actions and of the involved key signaling players that are discussed in this review.

Straub 2011; Vucic 2008). MTAs have been shown to affect these families of pro- and anti-apoptotic proteins (Rovini et al. 2011). Furthermore, the STAT family of transcription factors as well as the serine kinase Raf is influenced by microtubule binding compounds.

4.1. Bcl-2 family

The Bcl-2 family comprises pro-apoptotic (Bim, Bad, Bid, Noxa *etc.*) as well as anti-apoptotic (Bcl-2, Bcl-x) members (Antonsson et al. 2000). As Bcl-2 proteins play important roles through regulation of mitochondrial processes, such as release of pro-apoptotic molecules, and mitochondria are known to interact with microtubules (Esteve et al. 2007; Rovini et al. 2011) it seems likely that microtubule damage affects the mitochondrial activation and the onset of the apoptotic machinery. On the one hand, Bcl-2 has been attributed as "guardian of microtubule dynamics" (Haldar et al. 1997). Overexpression of Bcl-2 or Bcl-x has been shown by our group (yet unpublished data) and others (Gajate et al. 2000; Tang et al. 1994) to inhibit apoptosis by MTAs without affecting induction of mitotic arrest suggesting that Bcl-2 and Bcl-x are able to block important signaling events triggered by the drugs between or independent of mitotic arrest. The mechanism behind this is not entirely clear. On the other hand, however, it is known that MTAs lead to an activation of the MAPK JNK, which then phosphorylates Bcl-2 (Fan et al. 2000; Feng et al. 2011). Sustained phospho-Bcl-2 loses its ability to bind pro-apoptotic Bax, which leads to increased free Bax levels and, subsequently, apoptosis. We and others have shown that MTAs, such as spongistatin, pretubulysin, or paclitaxel, induce phosphorylation of Bcl-2 resulting in the inactivation of its anti-apoptotic activity (Blagosklonny et al. 1999; Herrmann et al. 2012; Rothmeier et al. 2010; Schneiders et al. 2009). Furthermore, it has been shown that paclitaxel is able to cleave Bcl-2 turning it from anti-apoptotic to pro-apoptotic (Blagosklonny et al. 1999). Moreover, Bcl-2 has been shown to be an important target of spongistatin in pancreatic tumor progression and metastasis. Silencing of Bcl-2 reduces cell death of invasive pancreatic tumor cells as well as its migration (Rothmeier et al. 2010).

4.2. Bim (Bcl-2-interacting mediator of cell death)

Bim is a so called BH3 only protein, which executes a strong pro-apoptotic action by antagonizing most of the pro-survival Bcl-2 family members (Willis et al. 2005). Bim is an important linker of the cytoskeleton and the apoptotic machinery. Under physiological conditions Bim is bound to the microtubule network *via* the dynein light chain (LC8) and thus kept away from binding to other Bcl-2 members as well as to Mcl-1, another important pro-survival factor (Puthalakath et al. 1999). Compounds that depolymerize microtubules and, in consequence, disrupt the binding of Bim to the dynein light chain are apoptotic stimuli by freeing Bim to translocate to the mitochondria and releasing pro-apoptotic factors such as cytochrome c to the cytosol (Schneiders et al. 2009). Accordingly, the caspase cascade is initiated, which ultimately leads to apoptotic cell death. Interestingly, own data indicate that spongistatin (an innovative depolymerizing agent derived from a marine sponge) is able to disrupt the Bim/Mcl-1 complex. Mcl-1 functions as a reservoir for pro-apoptotic factors by binding proteins like Bim (Han et al. 2005; Schneiders et al. 2009).

4.3. Inhibitor of apoptosis proteins (IAPs)

The IAP gene family plays an important role in regulating apoptosis. Their common structural feature is a baculovirus IAP repeat (BIR) motif. In the context of cancer they have mainly been implicated in the suppression of apoptotic cell death inhibiting the major caspases 3, 7, and 9 (Fulda et al. 2012). Besides cIAP-1 and 2, especially XIAP as well as survivin attracted attention with respect to microtubule interaction. Survivin is localized to components of the mitotic spindle microtubules and has been reported to bind and stabilize microtubules, thereby preserving the integrity of interphase microtubules (Kanwar et al. 2011). Disruption of survivin microtubule interaction by tubulin antagonists results in increased caspase activation (Nakahara et al. 2011; Weng et al. 2009). Interestingly, it has been found that a series of novel taxanes show increased drug cytotoxicity and apoptosis in parallel with their inability to induce survivin (Sharifi et al. 2010). There are a couple of publications including our own, which show that agents such as spongistatin, which depolymerize microtubules, lead to a downregulation of XIAP protein expression resulting in an increased sensitivity towards cell death (Gagnon et al. 2008; Holt et al. 2011; Schyschka et al. 2008).

4.4. STAT3

STATs are transcription factors that are inactive in the cytoplasm and, once phosphorylated by the JAK family of kinases, dimerize and translocate to the nucleus where they modulate transcription of various genes. STAT3, one of the seven STAT family members, is considered as an oncogenic transcription factor activating genes responsible for proliferation, survival, and differentiation. STAT3 is overexpressed in many tumor entities known also to be responsive to MTAs, such as the *Vinca* alkaloids or taxanes. It has been shown that paclitaxel decreases the association between STAT3 and the microtubules resulting in both a decrease of phosphorylation of STAT3 and a block of STAT3-dependent gene expression finally leading to inhibition of proliferation and induction of cell death. Paclitaxel is not considered to be a direct kinase inhibitor rather abrogating the pivotal interaction between STAT3 and microtubules (Walker et al. 2011, 2010).

4.5. Raf-1

Raf-1 is a serine/threonine kinase that is activated upon the recruitment of Ras to the plasma membrane and is involved in cell proliferation signaling. Raf-1 has been shown to coimmunoprecipitate with Bcl-2 (Wang et al. 1994). Several studies report that paclitaxel-induced apoptosis is mediated by the loss of Bcl-2 function as a result of phosphorylation by Raf-1 (Blagosklonny et al. 1997, 1996). Raf-1 activation on the other hand requires a direct interaction of paclitaxel with tubulin (Blagosklonny et al. 1996). In this context, it is important to note that both serine/threonine kinases as well their counterparts, *i.e.* phosphatases, are associated with microtubules and, thus, are likely to be affected by MTAs (Komis et al. 2011; Sontag et al. 1995).

5. Inhibition of metastasis

MTAs have been reported not only to inhibit tumor growth but also metastasis (Herrmann et al. 2012; Rothmeier et al. 2010; Schnaeker et al. 2004). Cell migration and invasion are fundamental processes in cancer metastasis. Hereby a dynamic interaction between the tumor cells and the extracellular matrix (ECM) as well as proteolytic remodeling of ECM takes place (Geho et al. 2005; Mehlen et al. 2006). Matrix metalloproteinases (MMPs) cleave and degrade ECM microstructures and enable cancer cells to enter blood and lymphatic vessels. Thus, MMPs are targets for anti-metastatic therapy (Deryugina et al. 2006). Inhibition of MMP activity is a major goal and, in this context, it has been shown that MMPs need to be transported within the cell in order to get secreted. In melanoma cells it has been shown that storage and transport of MMPs is conducted in small cytoplasmic vesicles that are associated with both, microtubules and kinesin, a molecular motor protein (Schnaeker et al. 2004). Both, microtubule depolymerizing agents (*e.g.* spongistatin) and microtubule stabilizer have been reported to affect MMP activity. Expression of MMP-9, a major gelatinase, is strongly reduced by microtubule depolymerization (low dose spongistatin), which goes in parallel with a significant reduction of metastasis in an orthotopic mouse model of pancreatic cancer (Rothmeier et al. 2010). Low dose paclitaxel on the other hand has shown to inhibit exocytosis of MMP-2 and MMP-9 in melanoma cells and in consequence invasion of melanoma (Schnaeker et al. 2004).

6. Inhibition of tumor angiogenesis

Tumor angiogenesis is the development of new tumor blood vessels out of existing ones. Solid tumors depend on a new own vasculature to grow beyond approx. 1–2 mm^3. Endothelial cells are the pivotal players in angiogenic processes. It is obvious that MTAs can inhibit endothelial cell proliferation by blocking mitosis. However, a large number of studies—of which we can only mention a few—has documented that MTAs can also interfere with mitosis-independent angiogenic events, *e.g.* mainly with endothelial migration and tube formation: In 1996, the first report in this regard demonstrated that paclitaxel inhibits angiogenic processes in cultured endothelial cells and *in vivo* (Belotti et al. 1996). This was not linked to any cytotoxicity, since the concentrations necessary for these effects were lower than that needed to affect endothelial cell proliferation. Also non-toxic concentrations of vinblastine showed anti-angiogenic effects *in vitro* (inhibition of endothelial chemotaxis, spreading, secretion of matrix metalloproteinases, tube formation) and also *in vivo* (Vacca et al. 1999). Another study showed that subtoxic concentrations of docetaxel and epothilone B reduce endothelial cell migration and tube formation by inhibition of the small GTPases Rac1 and Cdc42 (Bijman et al. 2006). Interestingly, docetaxel was also demonstrated to attenuate endothelial cell migration by inducing the proteasomal degradation of Hsp90 upon dissociation of Hsp90 from tubulin (Murtagh et al. 2006). This prevented signaling from the focal adhesions and also integrin activation. Our own group found that spongistatin 1 effectively blocks migration (directed as well as undirected), tube formation, and endothelial cell sprouting from mouse aortic rings (Rothmeier et al. 2009). Regarding the underlying molecular mechanisms, spongistatin 1 inhibited the translocation of the protein kinase C (PKC) α to the membrane and, thus, blocked the activation of downstream targets. Moreover, also the myxobacterial MTA tubulysin was reported to exhibit an anti-angiogenic potential in the typical *in vitro* assays (Kaur et al. 2006). Most recently, we investigated the effect of pretubulysin, a precursor of tubulysin, and of simplified derivatives on angiogenic processes *in vitro* and *in vivo* and could prove that this MTA has strong anti-angiogenic properties (Rath et al. 2012).

7. Vascular disrupting activity

The concept of vascular disruption as a promising strategy for tumor treatment came up in the 1980 s, when vessel occlusion was found to irreversibly damage the tumor capillary network leading to tumor regression in mice (Denekamp et al. 1983). This concept has been followed up and a large num-

ber of compounds have been developed in the recent years that exhibit vascular disrupting activity, which means that they induce a rapid and selective collapse of the existing tumor vasculature, leading to the blockage of blood flow followed by tumor necrosis (Tozer et al. 2005). These compounds are called vascular disrupting agents (VDAs) and most of them are currently tested in clinical trials (Hollebecque et al. 2012). MTAs represent the largest subclass of VDAs with the family of combretastatins—stilbenes isolated from the shrub *Combretum caffrum*—as the most prominent representative (Kanthou et al. 2009). The derivative combretastatin A-4 3-O-phosphate (CA4P, fosbretabulin, Zybrestat™) has attracted the largest attention and is, thus, in very advanced stages of clinical testing. Further relevant combretastatin derivatives are AVE8062 (ombrabulin) and OXi4503 (CA41P). Beyond the combretastatins, also a variety of very diverse (mostly heterocyclic) MTAs exist and are clinically evaluated, *e.g.* NPI-2358 (plinabulin), TZT-1027 (soblidotin), ABT-751, and also dolastatin 10.

All these agents selectively disrupt the tubulin cytoskeleton of tumor endothelial cells. The selectivity towards the tumor vasculature is only rudimentarily understood to date. The most accepted view is that—in contrast to the normal, well-organized vessel network of healthy tissue—tumor vessels are very immature, irregular, and highly disorganized due to their rapid development induced by overexpression of angiogenic factors and, therefore, are more easily vulnerable by VDAs (Siemann 2011). Regarding the underlying molecular mechanisms of action, the lead VDA CA4P was found to strongly increase endothelial permeability, to disrupt the VE-cadherin/β-catenin/Akt signaling pathway (Vincent et al. 2005), to induce RhoA activation and F-actin stress fiber formation (Kanthou et al. 2002), and to cause endothelial apoptosis (Ding et al. 2011). Interestingly, our own (yet unpublished) *in vivo* and *in vitro* data on the effects of the MTA pretubulysin show that this compound is a promising new VDA that rapidly enhances endothelial permeability, causes VE-cadherin and claudin-5 disassembly, and activates p38 MAPK, but does not cause apoptosis. Despite all this knowledge, the precise molecular events linking the disruption of microtubules with all the mentioned pathways is still a black box asking for further investigations.

8. Conclusion

MTAs are—and will remain—of utmost importance in curative and palliative cancer therapy. However, their mode of action thought to underlie their clinical efficacy, namely inhibition of mitosis due to interference with the spindle apparatus, has to be revised: Their interphase actions, comprising mitosis-independent induction of cell death, inhibition of metastasis and tumor angiogenesis, and vascular-disrupting activity, are the mechanistic basis for their success. Thus, research on tubulin binders is not old-fashioned, quite the contrary, microtubules are an ever young and attractive therapeutical drug target.

Acknowledgements: Verena Kretzschmann and Stefan Zahler kindly provided the images in Fig. 1 gained by immunocytochemistry and confocal laser scanning microscopy.

References

Antonsson B, Martinou JC (2000) The Bcl-2 protein family. Exp Cell Res 256: 50–57.

Belotti D, Vergani V, Drudis T, Borsotti P, Pitelli MR, Viale G, Giavazzi R, Taraboletti G (1996) The microtubule-affecting drug paclitaxel has antiangiogenic activity. Clin Cancer Res 2: 1843–1849.

Bijman MN, van Nieuw Amerongen GP, Laurens N, van Hinsbergh VW, Boven E (2006) Microtubule-targeting agents inhibit angiogenesis at subtoxic concentrations, a process associated with inhibition of Rac1 and Cdc42 activity and changes in the endothelial cytoskeleton. Mol Cancer Ther 5: 2348–2357.

Blagosklonny MV, Chuman Y, Bergan RC, Fojo T (1999) Mitogen-activated protein kinase pathway is dispensable for microtubule-active drug-induced Raf-1/Bcl-2 phosphorylation and apoptosis in leukemia cells. Leukemia 13: 1028–1036.

Blagosklonny MV, Giannakakou P, el-Deiry WS, Kingston DG, Higgs PI, Neckers L, Fojo T (1997) Raf-1/bcl-2 phosphorylation: a step from microtubule damage to cell death. Cancer Res 57: 130–135.

Blagosklonny MV, Schulte T, Nguyen P, Trepel J, Neckers LM (1996) Taxol-induced apoptosis and phosphorylation of Bcl-2 protein involves c-Raf-1 and represents a novel c-Raf-1 signal transduction pathway. Cancer Res 56: 1851–1854.

Denekamp J, Hill SA, Hobson B (1983) Vascular occlusion and tumour cell death. Eur J Cancer Clin Oncol 19: 271–275.

Deryugina EI, Quigley JP (2006) Matrix metalloproteinases and tumor metastasis. Cancer Metastasis Rev 25: 9–34.

Ding X, Zhang Z, Li S, Wang A (2011) Combretastatin A4 phosphate induces programmed cell death in vascular endothelial cells. Oncol Res 19: 303–309.

Esteve MA, Carre M, Braguer D (2007) Microtubules in apoptosis induction: are they necessary? Curr Cancer Drug Targets 7: 713–729.

Fan M, Goodwin M, Vu T, Brantley-Finley C, Gaarde WA, Chambers TC (2000) Vinblastine-induced phosphorylation of Bcl-2 and Bcl-XL is mediated by JNK and occurs in parallel with inactivation of the Raf-1/MEK/ERK cascade. J Biol Chem 275: 29980–29985.

Feng R, Li S, Lu C, Andreas C, Stolz DB, Mapara MY, Lentzsch S (2011) Targeting the microtubular network as a new antimyeloma strategy. Mol Cancer Ther 10: 1886–1896.

Fulda S, Vucic D (2012) Targeting IAP proteins for therapeutic intervention in cancer. Nat Rev Drug Discov 11: 109–124.

Gagnon V, Van Themsche C, Turner S, Leblanc V, Asselin E (2008) Akt and XIAP regulate the sensitivity of human uterine cancer cells to cisplatin, doxorubicin and taxol. Apoptosis 13: 259–271.

Gajate C, Barasoain I, Andreu JM, Mollinedo F (2000) Induction of apoptosis in leukemic cells by the reversible microtubule-disrupting agent 2-methoxy-5-(2',3',4'-trimethoxyphenyl)-2,4,6-cycloheptatrien-1-one: protection by Bcl-2 and Bcl-X(L) and cell cycle arrest. Cancer Res 60: 2651–2659.

Geho DH, Bandle RW, Clair T, Liotta LA (2005) Physiological mechanisms of tumor-cell invasion and migration. Physiology (Bethesda) 20: 194–200.

Haldar S, Basu A, Croce CM (1997) Bcl2 is the guardian of microtubule integrity. Cancer Res 57: 229–233.

Han J, Goldstein LA, Gastman BR, Rabinovitz A, Rabinowich H (2005) Disruption of Mcl-1.Bim complex in granzyme B-mediated mitochondrial apoptosis. J Biol Chem 280: 16383–16392.

Herrmann J, Elnakady YA, Wiedmann RM, Ullrich A, Rohde M, Kazmaier U, Vollmar AM, Muller R (2012) Pretubulysin: from hypothetical biosynthetic intermediate to potential lead in tumor therapy. PLoS One 7: e37416.

Hollebecque A, Massard C, Soria JC (2012) Vascular disrupting agents: a delicate balance between efficacy and side effects. Curr Opin Oncol 24: 305–315.

Holt SV, Brookes KE, Dive C, Makin GW (2011) Down-regulation of XIAP by AEG35156 in paediatric tumour cells induces apoptosis and sensitises cells to cytotoxic agents. Oncol Rep 25: 1177–1181.

Kanthou C, Tozer GM (2002) The tumor vascular targeting agent combretastatin A-4-phosphate induces reorganization of the actin cytoskeleton and early membrane blebbing in human endothelial cells. Blood 99: 2060–2069.

Kanthou C, Tozer GM (2009) Microtubule depolymerizing vascular disrupting agents: novel therapeutic agents for oncology and other pathologies. Int J Exp Pathol 90: 284–294.

Kanwar JR, Kamalapuram SK, Kanwar RK (2011) Targeting survivin in cancer: the cell-signalling perspective. Drug Discov Today 16: 485–494.

Kaur G, Hollingshead M, Holbeck S, Schauer-Vukasinovic V, Camalier RF, Domling A, Agarwal S (2006) Biological evaluation of tubulysin A: a potential anticancer and antiangiogenic natural product. Biochem J 396: 235–242.

Kelly PN, Strasser A (2011) The role of Bcl-2 and its pro-survival relatives in tumourigenesis and cancer therapy. Cell Death Differ 18: 1414–1424.

Komis G, Illes P, Beck M, Samaj J (2011) Microtubules and mitogen-activated protein kinase signalling. Curr Opin Plant Biol 14: 650–657.

Komlodi-Pasztor E, Sackett D, Wilkerson J, Fojo T (2011) Mitosis is not a key target of microtubule agents in patient tumors. Nat Rev Clin Oncol 8: 244–250.

Mehlen P, Puisieux A (2006) Metastasis: a question of life or death. Nat Rev Cancer 6: 449–458.

Murtagh J, Lu H, Schwartz EL (2006) Taxotere-induced inhibition of human endothelial cell migration is a result of heat shock protein 90 degradation. Cancer Res 66: 8192–8199.

Nakahara T, Yamanaka K, Hatakeyama S, Kita A, Takeuchi M, Kinoyama I, Matsuhisa A, Nakano K, Shishido T, Koutoku H, Sasamata M (2011) YM155, a novel survivin suppressant, enhances taxane-induced apoptosis and tumor regression in a human Calu 6 lung cancer xenograft model. Anticancer Drugs 22: 454–462.

Ola MS, Nawaz M, Ahsan H (2011) Role of Bcl-2 family proteins and caspases in the regulation of apoptosis. Mol Cell Biochem 351: 41–58.

Puthalakath H, Huang DC, O'Reilly LA, King SM, Strasser A (1999) The proapoptotic activity of the Bcl-2 family member Bim is regulated by interaction with the dynein motor complex. Mol Cell 3: 287–296.

Rath S, Liebl J, Furst R, Ullrich A, Burkhart J, Kazmaier U, Herrmann J, Muller R, Gunther M, Schreiner L, Wagner E, Vollmar A, Zahler S (2012) Anti-angiogenic effects of the tubulysin precursor pretubulysin and of simplified pretubulysin derivatives. Br J Pharmacol 167: 1048–1061.

Rothmeier AS, Ischenko I, Joore J, Garczarczyk D, Furst R, Bruns CJ, Vollmar AM, Zahler S (2009) Investigation of the marine compound spongistatin 1 links the inhibition of PKCalpha translocation to nonmitotic effects of tubulin antagonism in angiogenesis. FASEB J 23: 1127–1137.

Rothmeier AS, Schneiders UM, Wiedmann RM, Ischenko I, Bruns CJ, Rudy A, Zahler S, Vollmar AM (2010) The marine compound spongistatin 1 targets pancreatic tumor progression and metastasis. Int J Cancer 127: 1096–1105.

Rovini A, Savry A, Braguer D, Carre M (2011) Microtubule-targeted agents: when mitochondria become essential to chemotherapy. Biochim Biophys Acta 1807: 679–688.

Schnaeker EM, Ossig R, Ludwig T, Dreier R, Oberleithner H, Wilhelmi M, Schneider SW (2004) Microtubule-dependent matrix metalloproteinase-2/matrix metalloproteinase-9 exocytosis: prerequisite in human melanoma cell invasion. Cancer Res 64: 8924–8931.

Schneiders UM, Schyschka L, Rudy A, Vollmar AM (2009) BH3-only proteins Mcl-1 and Bim as well as endonuclease G are targeted in spongistatin 1-induced apoptosis in breast cancer cells. Mol Cancer Ther 8: 2914–2925.

Schyschka L, Rudy A, Jeremias I, Barth N, Pettit GR, Vollmar AM (2008) Spongistatin 1: a new chemosensitizing marine compound that degrades XIAP. Leukemia 22: 1737–1745.

Sharifi N, Qi J, Bane S, Sharma S, Li R, Robey R, Figg WD, Farrar WL, Kingston DG (2010) Survivin is not induced by novel taxanes. Mol Pharm 7: 2216–2223.

Siemann DW (2011) The unique characteristics of tumor vasculature and preclinical evidence for its selective disruption by Tumor-Vascular Disrupting Agents. Cancer Treat Rev 37: 63–74.

Sontag E, Nunbhakdi-Craig V, Bloom GS, Mumby MC (1995) A novel pool of protein phosphatase 2A is associated with microtubules and is regulated during the cell cycle. J Cell Biol 128: 1131–1144.

Straub CS (2011) Targeting IAPs as an approach to anti-cancer therapy. Curr Top Med Chem 11: 291–316.

Tang C, Willingham MC, Reed JC, Miyashita T, Ray S, Ponnathpur V, Huang Y, Mahoney ME, Bullock G, Bhalla K (1994) High levels of p26BCL-2 oncoprotein retard taxol-induced apoptosis in human pre-B leukemia cells. Leukemia 8: 1960–1969.

Tozer GM, Kanthou C, Baguley BC (2005) Disrupting tumour blood vessels. Nat Rev Cancer 5: 423–435.

Vacca A, Iurlaro M, Ribatti D, Minischetti M, Nico B, Ria R, Pellegrino A, Dammacco F (1999) Antiangiogenesis is produced by nontoxic doses of vinblastine. Blood 94: 4143–4155.

Vincent L, Kermani P, Young LM, Cheng J, Zhang F, Shido K, Lam G, Bompais-Vincent H, Zhu Z, Hicklin DJ, Bohlen P, Chaplin DJ, May C, Rafii S (2005) Combretastatin A4 phosphate induces rapid regression of tumor neovessels and growth through interference with vascular endothelial-cadherin signaling. J Clin Invest 115: 2992–3006.

Vucic D (2008) Targeting IAP (inhibitor of apoptosis) proteins for therapeutic intervention in tumors. Curr Cancer Drug Targets 8: 110–117.

Walker SR, Chaudhury M, Frank DA (2011) STAT3 Inhibition by microtubule-targeted drugs: dual molecular effects of chemotherapeutic agents. Mol Cell Pharmacol 3: 13–19.

Walker SR, Chaudhury M, Nelson EA, Frank DA (2010) Microtubule-targeted chemotherapeutic agents inhibit signal transducer and activator of transcription 3 (STAT3) signaling. Mol Pharmacol 78: 903–908.

Wang HG, Miyashita T, Takayama S, Sato T, Torigoe T, Krajewski S, Tanaka S, Hovey L, 3rd, Troppmair J, Rapp UR, et al. (1994) Apoptosis regulation by interaction of Bcl-2 protein and Raf-1 kinase. Oncogene 9: 2751–2756.

Weng D, Song X, Xing H, Ma X, Xia X, Weng Y, Zhou J, Xu G, Meng L, Zhu T, Wang S, Ma D (2009) Implication of the Akt2/survivin pathway as a critical target in paclitaxel treatment in human ovarian cancer cells. Cancer Lett 273: 257–265.

Willis SN, Adams JM (2005) Life in the balance: how BH3-only proteins induce apoptosis. Curr Opin Cell Biol 17: 617–625.

Yue QX, Liu X, Guo DA (2010) Microtubule-binding natural products for cancer therapy. Planta Med 76: 1037–1043.