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THE ROLE OF MICROTUBULES IN PANCREATIC CANCER: THERAPEUTIC PROGRESS

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ABSTRACT

Pancreatic cancer has extremely low prognosis, which is a direct result of its high aggressiveness and invasiveness as well as late diagnosis and lack of effective therapies. One of the most crucial drugs with highly optimistic clinical potential to join the fight against cancer are considered to be microtubule-targeting agents. Almost each type acts differently on cancer cells, but with one common and significant result, which is inhibition of cancer cells. We review here functions of microtubule cytoskeletal proteins in tumor cells and comprehensively examine effects of microtubule-targeting agents on pancreatic carcinoma.

KEYWORDS: pancreatic cancer, pancreatic ductal adenocarcinoma, microtubule-targeting agents, microtubules, literature review.

INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is a devastating malignant tumor that ranks fourth leading cause of tumor-related death worldwide with a 5-year survival rate of 6-7%.[1-3] PDAC is the most common type of pancreatic cancer (PC), amounting to over 90% of this malignancy.[4] In 2016, the number of new cases was estimated to be 53,000, and number of deaths was as high as 42,000 in the United States alone.[5] At present PDAC is the 12th most commonly diagnosed malignancy worldwide. Although the incidence of PDAC is 8–12.5/100 000 males and 6–7/100 000 females, the rate continues to increase year by year.[6] Such low survivability in PDAC is influenced by its high aggressiveness and invasiveness as well as late diagnosis and lack of effective therapies. Radical surgery is considered to be the first-line treatment of early-stage PDAC.[7] However, even upon diagnosing PDAC at an early stage, only 9.7% of patients can receive surgical treatment. Most of the patients (over 85%) are diagnosed with advanced-stage PDAC, thus losing even the slim opportunity for surgery. For such patients, chemotherapy remains the only treatment option.[5,8] If the diagnostics of PDAC are not to improve to make the diagnosis at an early stage and implementation of sufficient treatment possible, it is expected to become the 2nd cause of mortality among all malignancies by the end of the decade.[9]

In most cases, chemotherapy drugs prolong median survival by less than 12 months.[10] One of the reasons for the poor efficacy of chemotherapy is the clear connective tissue response in the PDAC stroma, which is mainly manifested as abnormal or malfunctioning vascular system, a large number of infiltrating macrophages and proliferating and activated fibroblasts.[12] Dense stromal tissue impedes the infiltration of chemotherapy drugs.[12] PDAC cells are extremely resistant to chemotherapy drugs, owing this to abnormal gene expression, gene mutation, abnormal activation or inhibition of cell signaling pathways, tumor hypoxia, and abnormal microenvironment.[13] Logically, more effective drugs and treatment regimens targeted on PDAC will help to improve its clinical outcome.[14] Researches on increasing the survival rate of PDAC patients have been carried out for many years. The investigations were focused on exploring prognostic markers and novel mechanisms of carcinogenesis. In spite of efforts made, difference in median overall survival for PDAC was shown not to exceed a period of three months between 1986 and 2016.[15] Researchers require the illustration of novel molecular targets biologically connected to PDAC behavior and basis for alternative approaches to PDAC therapy. This review focuses on the functions of microtubule cytoskeletal proteins in tumor cells and comprehensively examines effects of microtubule-targeting agents on PDAC.

Microtubule structure

Microtubules are one of the three main components of the cytoskeleton They are, as the name suggests, tube-shaped protein polymers approximately 25 nm in diameter formed by the combination of α- and β-tubulin heterodimers (50 kDa each in size).[16,17] They are involved in a variety of essential cellular processes and functions such as construction and sustaining of cell shape, movement, and intracellular transport spindle formation during mitosis, ensuring accurate chromosome separation, cell division and signal propagation. Microtubules expand from the microtubule-organizing center located in the centrosome and interact with a variety of organelles including endoplasmic reticulum, Golgi apparatus, lysosomes, and mitochondria.[18,20]

The heterodimers of α- and β-tubulin continiously polymerize and connect in a head-to-tail fashion to form 13 protofilaments. These fibrils join in parallel completing a wall of the microtubule and forming a polar tube with an outer diameter of 24 nm, an inner diameter of 15 nm, and a wall thickness of 5 nm.[21] This polarity is attributable to the arrangement of the heterodimers subunits and results in different equilibrium constants at both ends of the microtubules and difference in their dynamics. As a result, one end (β-tubulin ringed (+) end) grows and shrinks more rapidly than the other (α-tubulin ringed (-) end) as the latter faces microtubule-organizing center.[22,24] Because now the two ends of the microtubule have a different growth (assembly) and shrinking (disassembly) speed, this eventually leads to dynamic instability of the microtubule assembly dynamics.[23,25]

Tubulin polypeptides are ubiquitous and abundant in all eukaryotic cells. They are composed of approximately 450 amino acids, which are divided into the N-terminal region, middle region, and carboxy-terminal (C-terminal) region.[26] α- and β- tubulins are about 36-42% homologous but at the same time very different from each other because of extremely different sequences in their C-terminal tail. Proteins in the C-terminal tail comprise a highly disorganised peptide consisting of 18–24 amino acids. C-terminal tail is a target for post-translational alterations and mediated synergies with a variety of proteins that are specific to only one certain tubulin isotype.[27,28] They can also interact with microtubule-associated proteins to regulate microtubule assembly.[29] The N-terminals serve as guanosine-50-triphosphate (GTP) binding sites of α- and β-tubulins. The middle region is has a rigid and ball-shaped appearance and maintains stability of the microtubule.[30]

Microtubule dynamics

The main feature of microtubules is their dynamics, they constantly shrink and expand by reversible connection and disconnection of α/β-tubulin heterodimers at both ends.[31] Unique structure of microtubules makes the dynamics of tubulin heterodimer release and addition slower at the (−) end and significantly quicker at the (+) end.[32,33] The shrinking phase of microtubule is called "catastrophe" and defined as transition from lengthening to shrinking period at the (−) end of the microtubule.[34] Conversion from shrinking to lengthening period at the (+) end is known as the growth phase, or "rescue". A microtubule that does not undergo transition between these two states dies. The growth and shrinking phases frequencies, and catastrophe and rescue rates produce the dynamics of microtubules instability.[35,36]

Since the α-tubulin is located at the stable non-exchangeable site, the growth and shrinking of microtubules is regulated by the exchangeable site at the β-tubulin (+) end through addition and replacement of tubulin dimers. The process of growth (polymerization) and shrinking (depolymerization) is driven by the adhesion, hydrolysis, and transfer of guanosine triphosphate (GTP) on the GTP-bound β-tubulin site.[37,38] GTP hydrolysis is crucial for shift between commutative phases of growth and shrinking divided by "catastrophe" and "rescue".[17,39] As long as GTP is bound to and capped to the (+) end of the microtubule, its growth will continue. But α/β-tubulin dimer addition is slower than GTP hydrolysis in the cap. Such stochastic assembly and disassembly is the primary cause of the dynamic instability in microtubules. Other than dynamic instability, microtubules demonstrate another kind of behavior, termed "treadmilling". It is characterized by net growth at one end of the microtubule and balanced net shrinkage at the opposite end. It results in an essential flow of tubulin subunits from the (+) end to the (-) end of the microtubule, which is particularly critical in mitotic spindles.[40,41]

GTP binds to microtubules and hydrolyzes quickly repeatedly changing slightly curved tubulin-GTP into more deeply curved tubulin guanosine diphosphate (GDP). At the same time, curved tubulin-GDP remains linear in shape and serves as a part of the outer wall of the microtubule. The growth process of microtubules is maintained by capped tubulin-GDP, and the loss of this capped structure leads to the rapid depolymerization of microtubules. When the structure is closed, the microtubule maintains a steady state of a certain length. Microtubule generates polarity through difference in polymerization rates at its end.[42,43]

The disassembly of microtubules is accompanied by the formation of the new network of spindle microtubules that are much more dynamic than interphase microtubules (as high as 100 times!). This dramatic variation in the microtubule network produces mitotic spindles. Production of mitotic aster and centromeric microtubules requires stringent regulation of microtubule dynamics to assure individual chromosome attachment and segregation during cell division.[44] In addition, the natural dynamics of microtubule fibers permits conventional segregation of chromosomes. Failure to accurately attach or separate chromosomes initiates the arrest of the cell cycle in the mitotic checkpoint resulting

in the initiation of apoptosis. The microtubule dynamics change during the cell cycle and microtubules turnover slowly through the interphase. The duration of such turnover can last minutes or even hours depending on the cell type.[45,46]

There is a multitude of different regulatory proteins that play a role in microtubule structural stability: promoting microtubule stability proteins, such as (-) terminal combined with gamma-tubulin and gamma-tubulin compound protein (GCPs), lateral combination of microtubule-associated protein 2 (MAP2) and τ protein, (+) terminal-binding stabilizing microtubule proteins, such as beta TIPs (EB1 and CLIP170), etc. There are also microtubule-binding polymerizing or depolymerizing proteins such as cleaved enzymes (spastin and katanin), (+) terminal depolymerizing kinesin-13, and α/β- microtubule dimer stabilizing protein stathmin.[47,48] Microtubules also interact with proteins involved in intracellular transport (kinesins and dyneins), cell cycle and apoptosis regulatory proteins, including tumor suppressor protein p53, which connects directly to dynein and also interacts with Bcl-2, survivin and other prosurvival proteins.[49] However, the nature and function of these interactions between dividing cells and tumor cells are not clear and deserve further investigation.[50,51]

Isotypes of tubulin and their functions

Microtubules are composed of various tubulin isoforms, several of which are ancient in origin and key to aspects of microtubule function. In humans, microtubules are formed from various compounds of α-tubulin, β-tubulin, γ-tubulin, δ-, ε- and z- tubulin.[52] The heterodimers of α- and β-tubulin are the basic structural components that constitute microtubules and control various functions of microtubules. The members of the tubulin family differ from one another by sequences at C-terminal tail that functions as binding domain for MAPs.[53] There are eight different subtypes of α-tubulin that have been detected in the human body: α1A-Tubulin (TUBA1A), α1B-Tubulin (TUBA1B), α1C-Tubulin (TUBA1C), α3C-Tubulin (TUBA3C), α3E-Tubulin (TUBA3E), α4A-Tubulin (TUBA4A), α8-Tubulin (TUBA8), α-like 3-Tubulin (TUBAL3) and it is still under research. Another major component of the microtubule is β–tubulin, it hasten subtypes of β–tubulin, which areβI-Tubulin (TUBB), βII-Tubulin (TUBB2A, TUBB2B), βIII-Tubulin (TUBB3), βIVa-Tubulin (TUBB4), βIVb-Tubulin (TUBB2C), βV-Tubulin (TUBB6), βVI-Tubulin (TUBB1).[54,55] The γ -microtubules are mainly located at the (-) end of the microtubule (microtubule-organizing center) in the centrosome, they promote nucleation of intracellular microtubules and control mitotic spindle replication. Gamma-tubulin combined with accessory proteins in the cytoplasm form the γ-tubulin ring complex (TuRC)that appears as the platform for microtubule assembly. δ-tubulin, the fourth family of the tubulin superfamily, was recognized during the UNI3 gene mutants in the *Chlamydomonas* basal bodies. According to human genome database, it has approximately 40% of similarity in sequence to *Chlamydomonas*. Immunofluorescence studies exposed that δ-tubulin was found to interact with γ-tubulin at the centrosome on immunofluorescence images.[56] ε-tubulin has a similar sequence with other tubulin proteins. The studies also showed that ε-tubulin does not affect the function of the centrosome in terms of microtubules nucleation. Still there is a scarcity of information as to the interaction or synergy of γ-, δ- and ε-tubulin with microtubule.[57]

Altered expression of tubulin isotypes in cancer

As mentioned in the earlier paragraph, the tubulin dimer subunits are encoded by several α- and β-tubulin genes. Each gene is encoded into a different protein, so there exist a variety of tubulin isotypes. The expression of tubulin isotypes is changed between different organs and tissues. For example, TUBB is widely expressed in the body, whereas TUBB2A is confined to the nervous tissue and muscles, and TUBB3 is mostly expressed in neurons. Tubulin alterations are correlated with chemotherapy resistance, chemotherapeutic responses, tumor development, and tumor cell survival. Below, we review the tubulin isotypes involved in carcinogenesis and their association with drug resistance.

α-tubulin

The function of α-tubulin isotypes and their role in cancer require further investigation. Only several studies researched the expression of α-tubulin isotypes in cancer or normal tissues. The expression levels of α-tubulin isotypes are associated with sensitivity to anti-tubulin agents and poor prognosis in many types of cancer. Some studied found a correlation between high expression level of TUBA1B and poor prognosis in hepatocellular carcinoma and mantle cell lymphoma.[58,59] Also, upregulated expression of TUBA1C predicts poor prognosis and promotes proliferation and migration in hepatocellular carcinoma.[60] The expression of TUBA3C is associated with decreased response of ovarian cancer to paclitaxel.[61] TUBAL3K is overexpressed in five anaplastic carcinomas and related to thyroglobulin mRNA. High expression of kα-1-tubulin affected anaplastic carcinomas therapy with paclitaxel. Also, Δ2α-tubulin level is related to poor response to vinca alkaloid site in the treatment of advanced non-small cell lung carcinoma (NSCLC).[62]

β-tubulin

β-tubulin isotypes are investigated more comprehensively than α-tubulins. Increased expression of β-tubulin isotypes was described in a variety of tumors. Specimen analysis and clinical research determined that high production of different β-tubulin isotypes from βI to βVa, such as βI-, βII-, βIII-, βIVa-, and βV-tubulin, are associated with disease progression, aggressive clinical behavior, overall survival, poor patient outcome, and chemotherapy drug resistance.

Recent researches concluded that tumor aggressiveness, uncontrolled cell proliferation and malignant biological behaviors of tumor cells, such as infinite growth, invasion, metastasis and resistance to chemotherapeutic agents, are closely correlated with abnormal expression and distribution of β-tubulin isotypes. TUBB3 is the most commonly found highly expressed β-tubulin isotype that is related to cancer. Altered expression level of TUBB3 was observed in many human cancer cells, and its aberrant expression was found to go side-by-side with enhanced chemoresistance and poor prognosis in NSCLC, ovarian cancer, gastric cancer, breast cancer, and uterine serous carcinoma combine together[64,66] Moreover, increased expression of TUBB3 is associated with glioblastoma, colorectal cancer and PDAC.[67,68] High expression TUBB2 was shown to be correlated with decreased overall survival in colorectal cancer.[69] A handful of researches established a strong association between decreased TUBB2 expression and advanced stage of ovarian cancer, and resistance to taxane treatment in ovarian cancer.[70,71] Breast cancer cells were shown to have decreased response to docetaxel treatment in patients in case of high βI-tubulin expression.[66] Also, overexpression of TUBB4 is correlated with poor response of paclitaxel treatment in patients diagnosed with ovarian cancer and NSCLC.[72,73]

Tubulin in pancreatic cancer

Recent researches have determined the roles of TUBB2, 3 and 4 in PDAC. Immunohistochemical studies showed that those β-tubulin isotypes are more highly expressed in PC tissues in comparison with paracancerous tissues. Also, they are upregulated in PC cell lines and downregulated in normal human pancreatic cell lines (HPDE).[74,75]

βIII-tubulin

High-level expression of TUBB3 has been determined by immunohistochemical test in PDAC tissue specimens collected after surgical resection. The Western blot and RT-PCT showed difference in the expression of TUBB3 in PC cell lines and HPDE: TUBB3 was upregulated in the former and downregulated in the latter. It was demonstrated that knockdown of TUBB3 decreased the growth of cell colonies. The number of colonies significantly decreased with administration of chemotherapy drugs (gemcitabine, paclitaxel).[67] Knockdown of TUBB3 expression in PC cells leads to anchorage-independent and dependent cell growth, and related to enhanced anoikis (anchorage-independent apoptosis), strengthening the link between suppressed TUBB3 and initiation of apoptosis in PC cells. TUBB3 shRNA decreased tumorigenic potential, tumor growth and metastases of PC cells in a xenograft mouse model.[75]

βIV-tubulin

The βIV-Tubulin isotype includes two subtypes: tubulin βIVa (TUBB4) and βIVb-tubulin (TUBB2C). βIV-tubulin is highly expressed in PC cells, and, according to western blot analysis, its expression level was higher in all PDAC cell lines (MiaPaCa-2, HPAF-II and AsPC1) related to HPDE. TUBB2C plays an important role in regulating PC cells' anchorage-dependent growth and responsiveness to chemotherapeutic drugs.[75]

Notably, knockdown of TUBB2C largely influences PDAC cell growth and chemosensitivity. In particular, the knockdown of TUBB2C can enhance sensitivity of HPAF-II and AsPC1 cell lines to paclitaxel and gemcitabine, while no effect was observed in MiaPaCa-2 cell lines. TUBB2C may play a role in modulating chemoresistance in certain subsets of PDAC cell lines. For instance, knockdown of TUBB2C sensitizes all PDAC cell lines to vincristine by initiating apoptosis in tumor cells produced by vincristine. Further examination showed elevated sensitivity to other vinca alkaloid sites, including vinorelbine and vinblastine, following TUBB2C knockdown. Taken together, these data contribute to opening new possibilities for PDAC therapy to produce inhibitors against TUBB2C and vinca alkaloid site. Knockdown of TUBB2C had no effect on normal pancreatic HPDE cell proliferation, in other words, its anti-proliferative influences are highly specific to cancer cells. The results clearly showed that knockdown of βIVb-tubulin induces the ability of vinca alkaloid site to arrest mitosis and then induce apoptosis. In summary, TUBB2C has a high probability of becoming an object of thorough research of therapeutic targets that may increase sensitivity of PDAC cells to ligands binding to vinca alkaloid site.[74]

Therapeutic efficiency of a novel βIII/βIV-tubulin inhibitor (VERU-111)

Similarly to other cancers, tubulins play a significant role in the progression of PDAC. Among all tubulins, βIII and βIV isotypes are believed to have the strongest association with PDAC progression, metastasis and chemoresistance.[27,77] Therefore, it is necessary to find selective targeting of βIII and βIV tubulins that may improve therapeutic response of PDAC. Recently, a novel βIII and βIV inhibitor, VERU-111, was created. In fact, VERU-111 can efficiently suppress the growth of aggressive PC cells. qPCR and Western blot analysis demonstrated potent inhibitory properties of VERU-111 that arise from its ability to affect expression of all the β-tubulin isotypes.[78] Another study found that miR-200c was significantly restored in PDAC cells after VERU-111 administration ($p < 0.01$), whereas miR-200c inhibitor could decrease the effect of VERU-111 on the expression of TUBB3. In other words, VERU-111 most likely targets TUBB3 via miR-200c.[79] By causing cell cycle arrest in the G2/M phase, mechanism of action of VERU-111 is somewhat similar to other microtubule-targeting agents (MTAs). Due to arrest in the G2 phase, cancer cells can no longer repair DNA damage, so they move directly into M phase, making the G2/M checkpoint suitable target for anti-cancer drugs. VERU-111 also affects the expression of Cdc2, cyclin B1 and Cdc25C kinases.[80] Flow cytometry data showed that

VERU-111 induces apoptosis in PDAC cells via altering mitochondrial proteins (Bcl-xL, Bcl-2, Bax and Bad), and, additionally, can activate caspase-3, caspase-9 and cleavage of PARP essential in apoptotic pathway.[81] These outcomes suggest involvement of multiple apoptosis-related proteins in death of PDAC cells caused by VERU-111. The xenograft mouse model examinations confirm that VERU-111 (50 μg/mice) can effectively suppress tumor growth with concomitant interference of βI, βIII and βIV tubulins and restore miR-200c expression. Based on the data obtained, VERU-111 suppresses pancreatic tumor growth via influencing cell cycle arrest, restoration of miR-200c and inducing apoptosis of PDAC cells, and may be efficacious in PDAC treatment.[78]

Microtubules as targets in cancer chemotherapy

Because of their pivotal role of microtubules in the mitotic cell division, they have become one of the core approaches in cancer pharmacology and targeted therapy.[82] As the cell undergoes prophase, microtubules existing in the cytoplasm begin to depolymerize more rapidly.[83] This highly dynamic process is crucial for the assembly of the mitotic spindle, and prompt and complete segregation of chromosomes during cell division. In the following stage of division, spindle microtubules pull the sister chromatids from the equator to the two poles of the spindle (Fig. 1). End of mitosis is marked by depolymerization of spindle microtubules as they assemble back into cytoplasmic microtubules. The dynamic characteristics of the depolymerization and polymerization are necessary for cells to complete mitosis.[84]

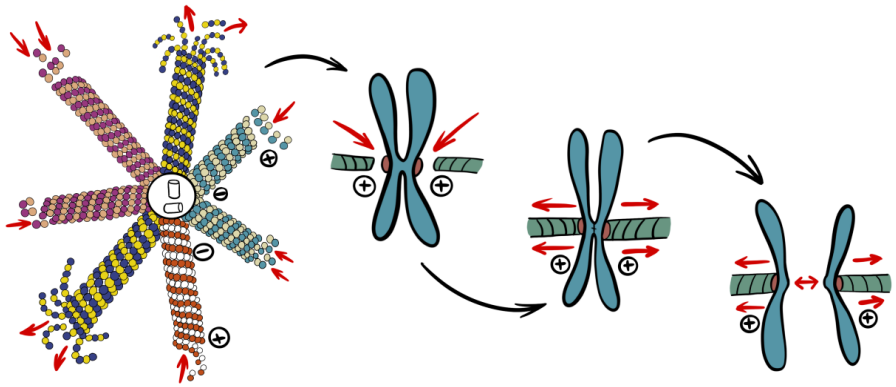

Fig. 1: Simplified role of microtubules in mitosis. Catastrophe rate of cytoplasmic microtubules increases to provide building blocks to different populations of spindle microtubules required for mitosis. Nuclear envelope breakdown allows spindle microtubules to attach to kinetochores of chromosomes. After chromosomes are aligned at equator, chromatids can finally segregate through depolymerization of attached microtubules and spindle pole movement.

If this cycle is interrupted, the cell will not enter mitosis, or cell division will be disrupted followed by mitotic arrest or division errors within mitotic sequence during anaphase, halted proliferation and cell death.[82] Impairment in dynamic behavior of microtubules will affect mitotic process of tumor cells and inhibit their growth or induce apoptosis. Therefore, microtubules are believed to be one of the most promising targets in combating cancer. In fact, most of the anti-angiogenic agents in clinical trials are MTAs. Microtubule inhibitors comprise highly effective class of anti-cancer drugs, and have been widely applied in the treatment of hematopoietic and solid tumors. Most of these MTAs are anti-mitotic agents that induce cell cycle arrest in the G2/M phase and produce irregular mitotic spindles.[85] They disrupt the structure of microtubules and inhibit cell proliferation by alternating polymerization dynamics of spindle microtubules.[77] There is a multitude of MTAs. Most can be classified functionally into 2 groups: microtubule-destabilizing agents (MDAs) and microtubule-stabilizing agents (MSAs).

Microtubule-destabilizing agents

Compounds that inhibit microtubule polymerization and reduce microtubule polymer bulk are known as MDAs. Agents in this group arrest the formation of mitotic spindles by acting on different structures of microtubules mainly colchicine or vinca alkaloid sites.[86]

1. Colchicines

Colchicine was originally isolated in 1820 by French chemists J.B. Caventou and P.S. Pelletier from Autumn Crocus (*Colchicum autumnale*), hence the name. Colchicine exhibits immunomodulatory properties at low concentrations, and is used primarily to alleviate the pain from gout. Its application in tumor treatment is limited largely by numerous side effects. However, its site of action – colchicine site – is used by the majority of MDAs and they gained enormous popularity in cancer treatment. Colchicine inhibits microtubule polymerization by binding to microtubule ends rather than to the soluble-tubulin pool. Colchicine binding site is located at the junction of the α-β subunit of the microtubule (Fig. 2), which is adjacent to the GTP binding site on the α-subunit.[87] Binding to β-tubulin

results in the original straight conformation bending causing steric hindrance between colchicine and α-tubulin. This binding first occurs on the α/β-tubulin dimer, which does not participate in the microtubules formation.[140] The stable complex formed at the end of microtubules significantly reduces the microtubule capacity to polymerize. Notably, colchicine can also induce microtubule depolymerization by inhibiting the interaction between the sides of microtubule fibrils.[56]

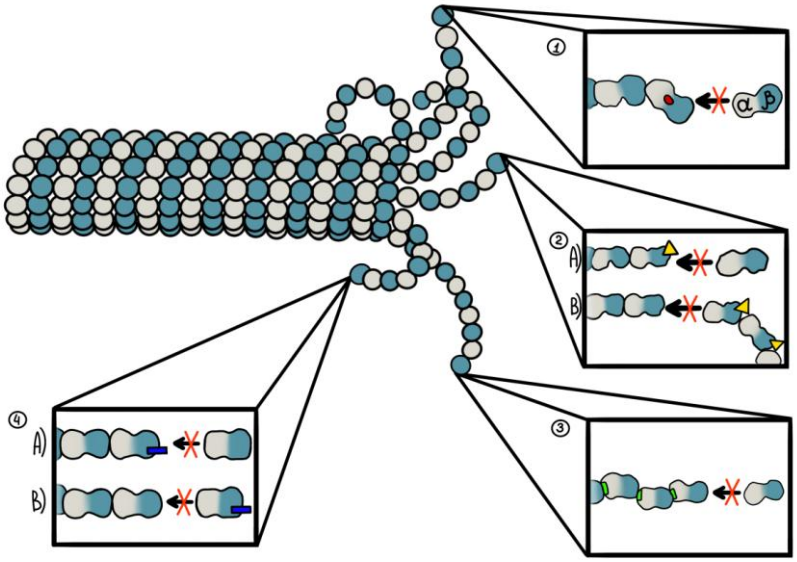

Fig. 2: Microtubule-destabilizing agents. 1. Colchicine site 2. Vinca alkaloid site 3. Pironetin site 4. Maytansine site.

2methoxyestradiol (2ME)

2methoxyestradiol (2ME) is derivative of mammalian endogenous estradiol. It can significantly inhibit proliferation of a variety of tumors, proliferation of endothelial cells *in vitro* and vascular proliferation *in vivo*. 2ME bind to the colchicine site and inhibit aggregation of microtubule and arrest cell cycle in the G2/M phase. New compounds are made by modifying structure of 2ME.[141,142] Some of those compounds displayed equally strong or even stronger inhibitory activity of tumor cell proliferation.[88]

Combretastatin

Combretastatin is found in *Combretum cafrum*, a tree growing in South Africa. Among colchicines site-binding compounds, combretastatin-A4 (CA-4) and combretastatin-A2 are the most efficacious. But CA-4 is an even stronger inhibitor than combretastatin A2. They both inhibit microtubule polymerization and mitosis, ultimately leading to apoptosis. High concentrations of combretastatin promote microtubule-dependent GTP hydrolysis, thereby completely inhibiting microtubule polymerization. Combretastatin displays a remarkable effect of inhibiting angiogenesis, interfering with tumor nuclei.[89,90]

Podophyllotoxin (PDT)

Podophyllotoxin (PDT) is a natural aryl tetrahydronaphthalene lignin compound isolated from *Polophyllum peltatum*. Although it was used in the treatment of constipation and gout, it also found its use in modern cancer therapy. PDT makes reconnection of DNA strands impossible through inhibiting DNA topoisomerase II. Consequently, arrest occurs in the G2 phase of the cell cycle. Two more low-toxic PDT derivatives, etoposide and teniposide, were synthesized.[91]

Chalcones

Chalcones were isolated from ferns, Myrtle evergreen plants such as *Calythropsis aurea*, *Piper aduncum* and *Fissistigma lanuginosum*. Trans-1-(2,5-dimethoxy)-3-[4-dimethylamino-phenyl]-2-methyl-2-propen-1 one (MDL) is a potent mitotic inhibitor that can rapidly and reversibly bind to the colchicine site on the –(+) end and inhibit the polymerization of the microtubule. MDL and podophyllotoxin both bind to the same site and display similar effect.[92]

Chamaecypanone C

Chamaecypanone C is a new compound mainly acting on the cytoskeleton. It blocks aggregation of tubulin by binding to the colchicine site. Chamaecypanone C induces apoptosis through caspase 8-fas/FasL pathway instead of mitochondria/caspase 9-dependent pathway like colchicine and paclitaxel. There are other agents or similar to chamaecypanone C, for example, 2-aroyl-4-phenyl-5-hydroxybenzofurans that act on the same site. They can arrest the cell cycle of tumor cells in the G2/M phase and induce apoptosis.[93] Phenyl 4-(2-oxoimidazolidin-1-yl) benzenesulfonates (PIB SOS) are useful in tumors with resistance to colchicine, paclitaxel,

vinblastine and overexpression of P-glycoprotein. These compounds deform and destroy cytoskeleton facilitating cell death. 2, 6-dinitro-4-(trifluoromethyl), phenoxycylaldoxime derivatives can also inhibit aggregation of microtubule, leading to decreased tumor cell proliferation.[94]

2. Vinca alkaloids

Vinca alkaloids are isolated from the Vincarosea plant (*Catharanthus roseus*). These compounds have a wide range of management of various seemingly unrelated diseases from diabetes and hypertension to cancer. Vinca alkaloid drugs occupy one large site on tubulin can inhibit mitotic progression, result in apoptosis and cell death. These drugs include vinblastine, vindesine, vinorelbine, vinflunine, and vincristine.

The binding site of vinca alkaloids is located near the GTP binding site of β-tubulin, acting like a wedging between microtubule protein molecules to prevent assembly of new microtubule protein dimer onto microtubule.[95] Experimental studies determined that vinca alkaloids mainly bind to amino acid residues at the 175-213 position of β-tubulin. They induce microtubules depolymerization at high concentrations. Incomplete crystallization of microtubules occurs at > 10 mmol/L concentration (para crystals). Within the range of clinical use, vinblastine can bind to the (+) end of the microtubule. Because of this, vinblastine drugs are sometimes called "end poisons". Vincristine also affects microtubules depolymerization that leads to decreased microtubule dynamics, abnormal cell spindles, and blocked mitosis.[96]

Recently, researchers have found that the cyclic terpene synthase can be used to make vincristine from compounds found in medicinal herb catnip, which may improve the simplicity of its source. The drug eribulin mesylate (Halaven®), which acts on the vinca alkaloid site, was approved by the FDA in 2010 for the treatment of breast cancer.

Dolastatins

Dolastatins are endogenous short peptides extracted from *Dolabella auricularia*. The binding sites of dolastatins in microtubules are similar to those of vinblastine.[99] Dolastatin 10 is the most effective mitotic inhibitor, it can prevent cell mitosis by breaking down microtubules.

Cryptophycins

Cryptophycins are a class of macrolides acting on microtubules. They are isolated from cyanobacteria. Research established similarity between cryptophycins and vinblastines: they both bind to the same binding site,[143] but through different mechanism. Cryptophycin mainly bind to the end of microtubule growth.[144] They can induce the formation of a single ring oligomer of microtubule protein, hinder the formation of a double ring oligomer of GTP or GDP of the microtubule protein and consequently inhibit microtubule polymerization.[100]

3. Pironetin

Pironetin is obtained from the culture medium of *Streptomyces actinomycete* nk10958. It is unique in establishing a covalent bond with L-lysine at 352 positions exclusively located on α-tubulin, which is relatively stable and not as active as β-tubulin. It can substantially inhibit microtubule polymerization. It has potent activity against tumor cell proliferation, and is able to avoid production of drug-resistant tumor cells.[102,103]

4. Maytansine

Maytansine is another natural anti-cancer compound extracted from the higher plant *Maytenus ovatus*. Just like vincristine, it can inhibit proliferation of cancer cells, but at the same time very different from it regarding inhibition of microtubule polymerization. Binding site of maytansine was inhibited by vincristine, and the binding affinity of maytansine was much higher compared with vincristine.[97] This “interaction” between vinca alkaloids and maytansine made the scientists in the past believe that maytansine ligands bind to vinca alkaloid site rather than have its own.

5. Other compounds

Some compounds such as phomopsinA, isolated from *Phomopsis leptosomiformis*, not only inhibit the polymerization of microtubule but also nucleotide hydrolysis and microtubule-dependent transport. Some studies have shown that phomopsinA acts on the formation of microtubule helix polymers.[101]

Halichondrins and halistatins are marine microtubule inhibitors. Halichondrin B is a lactone polyether extracted from *Halichondria okadai*, and halistatin is harvested from another sponge - *Axinellidae axinella*. Halistatin affects the assembly of microtubule *in vitro* and nucleotide exchange. Halichondrin B is a noncompetitive inhibitor of the vinca alkaloid site.

Rhizoxin is a macrolide compound with good anti-cancer activity isolated from a fungus (*Rhizopus Chinensis*). In spite of completely different structure, it binds to the same maytansine site and inhibits microtubule formation as aforementioned drug. Anti-cancer properties of rhizoxin on humans and mice are higher than those of maytansine.[98]

Microtubule-stabilizing agents

The mechanism of action of this type of microtubule inhibitors is directly opposite of MDAs, but the end result is the same. They influence cell proliferation by inhibiting cell division and blocking the cell cycle in the G2/M phase, producing abnormal mitotic spindle afterwards and, finally, causing cancer cell death by triggering apoptosis. MSAs mainly promote polymerization of microtubules, make them unusually stable and increase their quantities in the cell.[104] Two main binding sites are illustrated on Fig. 3.

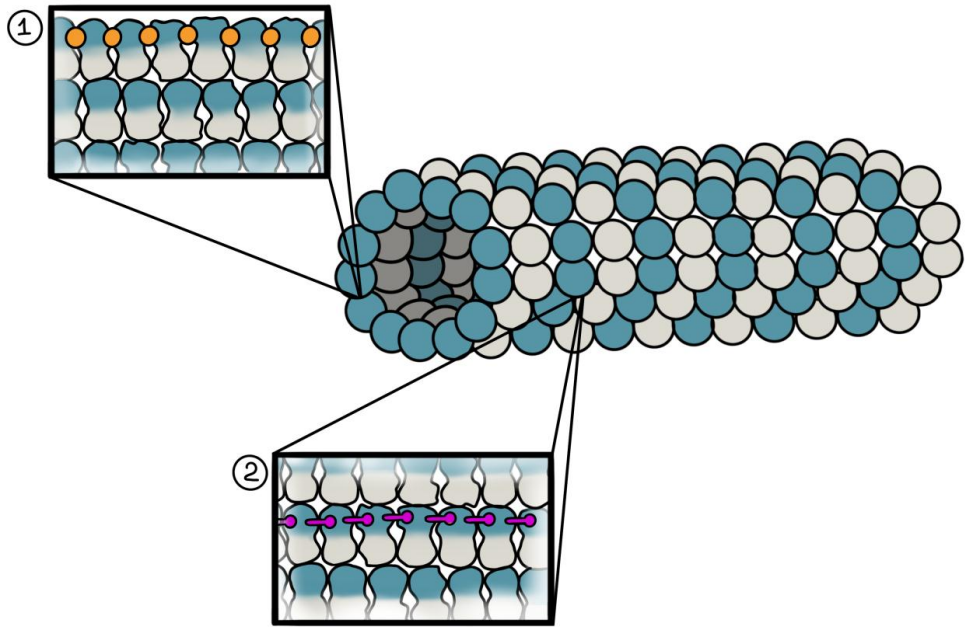

Fig. 3: Microtubule-stabilizing agents. 1. Taxane site 2. Peloruside A/Laulimalide site.

1. Taxanes

The representative drug of this group is paclitaxel (Taxol®). It easily binds to the assembled microtubules on the β-tubulin subunit. Generally, the process of microtubules polymerization requires GTP, but paclitaxel can promote tubulin polymerization without it. Paclitaxel promotes microtubule polymerization at low concentration and temperature without significantly rising polymer levels of the microtubule.[104, 105]

Epothilones

In past decades, epothilones have been under close investigation. It is a class of 16 membered rings extracted from *Sporangium Cellulosum* that share similar mechanism of action and overlapping yet completely different binding site with taxanes. Compared with paclitaxel, epothilone A is more simplistic in structure, better in water solubility and stronger in biological activity. In addition, paclitaxel-resistant tumor cells have no "defense" against epothilone, but it exert less cytotoxic effects compared with paclitaxel.[106]

Discodermolide

Discodermolide is a marine natural product isolated from a sponge *Discodermia dissolute*. It can promote microtubule protein aggregation. Results obtained from quantitative analysis indicate that discodermolide is more potent to induce microtubule aggregation than paclitaxel. To aggregate 10 μmol/L^{-1} of polymerized microtubule, IC-50 value of 23 μmol/$^{-1}$ of paclitaxel at room temperature is required, while only 3.2 μmol/L^{-1} of discodermolide is needed. Discodermolide binds to the taxane site so that the amount of taxol added will not affect discodermolide's binding. These results indicate that discodermolide binds to microtubules better than paclitaxel and is reasonable to act on or overlap with paclitaxel sites. The total synthesis of discodermolide and its structure-activity relationship has been thoroughly described, and many analogs have been synthesized. Although clinical studies have found that discodermolide is toxic, analogs are proper to become clinical candidates and enter clinical research.[109,110]

Dictyostatin

Dictyostatin was shown to influence microtubule polymerization *in vitro*. This causes spindle body production abnormalities, cell cycle in the G2/M phase stagnation and ultimately apoptosis. Dictyostatin is found to be more effective than paclitaxel in inhibiting the growth of drug-sensitive human cancer cells.[111]

(Z)-1-aryl-3-arylamino-2-propen-1-'ones

(Z)-1-aryl-3-arylamino-2-propen-1-one[10] compounds enhance microtubule stability and induce cell apoptosis via caspase family.[112] (Z)-1-(2-bromo-3,4,5-trimethoxyphenyl)-3-(3-hydroxy-4-methoxyphenino)-prop-2-en-1-one (10ae) can hinder cell proliferation in at least 20 tumor cell lines including drug-resistant tumor cell lines. Flow cytometry showed 10ae arrests cells undergoing G2/M phase.[50]

2. Peloruside A/Laulimalide

Peloruside A/Laulimalide (PLA/LAU) are anti-microtubule agents binding to the special site, named PEL/LAU, located on the β-tubulin. They are macrolides extracted from the sponges of *Mycale* and *Cacospongia* genera, respectively. Either display action very similar to that of paclitaxel, they both inhibit cancer cell proliferation and promote tubulin polymerization, but the rate of stabilizing tubulin is lower than in the latter.

Laurimalide induces the reorganization of the microtubule network, creating abnormality in spindles structure, as well as chromosome coiling and multinucleolate. Peloruside A demonstrates anti-proliferative properties in many cancer types. Laurimalide can induce the reorganization of the microtubule network, as well as creating abnormality in the structure of spindles, chromosome coiling and multinucleolate. What is of greater importance, laulimalide is proven to be effective in both epothilones and paclitaxel-resistant cancer cell lines with β-tubulin mutations on the taxane binding site and overexpression of PGP.[107,108]

3. Other compounds

Other compounds, such as jatrophane esters are macroditerpenoids extracted from *Euphorbia*. Jatrophone esters (J1-J3) can induce microtubule polymerization *in vivo*.[145] In addition, the degree of polymerization was far less stable than that induced by paclitaxel.

Ferulenol is an isopentenized coumarin compound isolated from the umbelliferous plant *Illicium verum Hoo*. Ferulenol has the same effect of promoting microtubule polymerization as paclitaxel.[146]

Evodiamine is the main component of *Evodia rutaecarpa (Juss) Benth*. Evodiamine affects proliferation and metastasis formation of tumor cells. It was found that evodiamine prolongs the binding of NCI/adrre of tubulin binding and significantly affects the balance between depolymerization and polymerization of microtubule.[147] Later study showed that evodiamine could stop cell division in the G2/M phase and enhance phosphorylation of c-raf and Bcl-2, initiating apoptosis.[148]

Fr182877, also known as cyclostreptin, is a bacterial metabolite obtained through the fermentation broth of 9885 *Streptomyces* in 2000. It promotes the polymerization of tubulin *in vitro* and cell cycle stagnation in the G2/M phase. It binds to different structures on taxane site than paclitaxel and shows less potent anti-proliferative activity than the latter *in vitro* and *in vivo*.[113]

Microtubule-destabilizing agents in pancreatic cancer

N-acetyl-O-methylcolchinol (NSC 51046)

Derivatives of colchicine have potent activity in many types of cancer cells, including PDAC cell lines. NSC 51046 was reported to halt mitosis by inhibiting microtubule polymerization (Table 1). It can hinder the cell cycle of PC cells triggering apoptosis. NSC 51046 inhibits tubulin polymerization at low doses and, strikingly, also promotes tubulin polymerization at higher doses. NSC 51046 induces apoptotic cell death in approximately 70% of both PC cell lines (PANC-1) and normal fibroblasts. Nonetheless, NSC 51046 displays non-selective properties and mild potent activity, preventing it from becoming a part of cancer-targeting therapy. Its analogs might prove different.[125]

Table 1: Microtubule-targeting agents in pancreatic cancer.

	Name	Origin	Anti-cancer properties	References
Taxane site	Paclitaxel	*Taxus brevifolia*	Probably p53 stimulation	[114], [115], [122], [139], [152]
	Nab-paclitaxel	Paclitaxel (*Taxus brevifolia*)	Probably p53 stimulation	[114-122]
	Epothilones	*Sorangium cellulose*	Apoptosis induction (probably Bcl-2 targeting)	[123], [124]
	10ae	Synthetic	Anti-proliferative, apoptosis induction (caspase family activation)	[112]
Colchicine site	NSC 51046	Colchicine (*Gloriosa superba*/*Colchicum autumnale*)	Apoptosis induction, anti-vascular	[125]
	UA62784	Synthetic	Anti-proliferative, apoptosis induction	[128], [129]
	Plinabulin	Synthetic (*Aspergillus ustus*)	Anti-proliferative	[130], [131]
	TH-484, TH-337, TH-494	Synthetic (indazole)	Anti-proliferative, anti-vascular	[132]
Vinca alkaloid site	DZ-2384	AB-5 (synthetic (*Diazona angulata*))	Anti-proliferative	[126], [127]

DZ-2384

DZ-2384 is a synthetic derivative of AB-5. AB-5 is long known to have anti-tumor activity in animal xenograft models. Its precursor is diazonamide A, another sponge-isolated compound. DZ-2384 synthesis is simplified and commercially scalable compared with earlier diazonamide analogs.[126] It raises microtubule rescue rate unlike vinorelbine, and the produced difference in microtubule dynamics is more notable than in other MDAs such as dolastatin 10, vincristine, etc.

DZ-2384 shows anti-tumor activity in PC xenograft models and other types of cancer in various models such as patient-derived xenograft model, genetically engineered mouse model with immunocompetent mice. DZ-2384 binds to the vinca alkaloid site of tubulin in a unique way producing higher anti-tumor properties and safety. The electron microscopy and X-ray crystallography showed that DZ-2384 modifies the curvature between tubulin dimers, thus straightening protofilaments. It enhances the rescue frequency, and, in spite of its limited effect on microtubules destabilization compared with vinorelbine, it is adequately sufficient to disrupt mitotic spindle formation. As a single agent alone, DZ-2384 has anti-tumor activity in MIA PaCa-2

model of PDAC. The experiment was conducted along with vinorelbine in two MIA PaCa-2 subcutaneous xenograft models of PDAC. The scientists reported that DZ-2384 induced complete neoplasm regression in the MIA-PaCa-2 xenograft model, and all the mice were cancer-free ~3 months after therapy (9 mg/m^2). Vinorelbine was also effective in both xenograft models, but at higher doses and for a shorter term. Importance of DZ-2384 also lies in its increased safety margin (more than 24-fold vs 0.7- to 1.0-fold in vinorelbine) in terms of weight loss, prognosis, bone marrow toxicity, and more than 13-fold in terms of neurotoxicity. Combination of DZ-2384 and gemcitabine was found to be more efficacious than gemcitabine monotherapy, which is the first-line treatment of patients with PDAC. DZ-2384 together with gemcitabine decreased tumor formation and progression with a higher response rate (68%) than combination of nab-paclitaxel and gemcitabine (53%) in Rgs16::GFP; KIC model. These results are indicative of DZ-2384 being a possible candidate for PDAC treatment and its potential in a wide range of other applications.[127]

UA62784

UA62784 is one of few compounds that selectively target PDAC cells. It is a novel highly potent microtubule inhibitor with enormous cytotoxicity whether used alone or with other MDAs. Actually, its cytotoxicity is additive with that of vinca alkaloids and may solve the problem of cancer cells resistance to MDAs.

The intrinsic tubulin tryptophan fluorescence experiments demonstrated the ability of UA62784 to bind to α- and β-tubulin dimers. UA62784 similarly to other MDAs compounds such as vinblastine, nocodazole and colchicines induced a fluorescence quenching upon binding to the α- and β-tubulin dimmers in intrinsic tubulin tryptophas fluorescence experiments. UA62784 displayed a high affinity of (27 ± 13 nM) for tubulin in a model with high affinity site with dissociation constant in the nanomolar range (Kd1) and of 142 ± 104 μM in a model with low affinity site with dissociation constant in the micromolar range (Kd2). Since Kd2 value is greater than Kd1 by more than 5000 times, the former could be left out for UA62784. Kd1 values for known microtubules anti-tumor agents such as colchicine (324 ± 36 nM), vinblastine (227 ± 45 nM) and nocodazole (259 ± 86 nM) show a roughly 10 times lower affinity of these drugs compared with UA62784. On the other hand, the affinity of second binding mode (Kd2) is 2-fold (colchicine and vinblastine) to 8-fold (nocodazole) higher than UA62784, suggesting possibility of existence of second binding site in this compound.

[3H]-colchicine experiment showed that the synergy of [3H]-colchicine with tubulin is reduced by the addition of raising doses of UA62784. The addition of 2 to 4 μM of UA62784 compound dismisses more than 60% of the tubulin heterodimers bound to [3H]-colchicine, which is similar to the outcome produced by 10 μM of colchicine. On the contrary, even high doses of vinblastine do not alter the interaction of [3H]-colchicine with tubulin. Taken together, this confirms that UA62784 directly combines with tubulin heterodimers at the colchicine-binding site and affects the quantity and amount of polymerized microtubulin *in vitro*.

Flow cytometry assay indicated that 20 nM of UA62784 for 12 hours increases the doubling in the G2/M phase from 21.5% ± 2.8% to 40.1% ± 1.1% in untreated HeLa cells. At a higher dose of 200 nM for 24 hours, UA62784 promoted the accumulation of phosphorylated histone H3, Cyclin B and MPM2. The presence of another mitotic marker, phosphorylated BubR1/BUB1B, strongly indicates that UA62784-treated tumor cells undergo mitotic arrest due to spindle assembly checkpoint (SAC) being "turned on". Moreover, β-tubulin staining showed that the administration of UA62784 promotes microtubule depolymerization in PC cells (Panc-1 cell lines). Finally, UA62784 is also appealing to structural changes and modeling due to its simplistic structure and properties.[128,129]

Plinabulin

Plinabulin is isolated from a fungal metabolite from *Aspergillus ustus*. It inhibits microtubule depolymerization. Recently, a combination treatment consisting of docetaxel and plinabulin has entered phase III trial for NSCLC therapy. Plinabulin has to be administered by intravenous injection due to its poor water-solubility. Synthetic derivatives of plinabulin, compounds 1 and 2, display activity against human BxPC-3 PC cell lines proliferation (inhibition percentage - >85% at 12.5 nM). These 2 derivatives can successfully suppress the activity of BxPC-3 PC cell line. Compound 2 with the IC50 value of 0.63 nM exhibited stronger anti-tumor activity than plinabulin at 4.28 nM.[130,131]

Other compounds

TH-482, TH-337 and TH-494 are lead compounds that belong to indazole-based microtubule inhibitors. They have potent anti-proliferative activity against PC cells (MIA PaCa-2 cell lines). TH-482 has the most potent anti-proliferative activity in 11 cell lines, including MIA PaCa-2 cell lines. TH-482 binds to the colchicine site of the microtubule, inhibits tubulin polymerization *in vitro* and leads to G2/M phase arrest. In addition to its effect on cell cycle, TH-482 exhibits vascular-disrupting activity *in vitro*. It hinders angiogenesis by endothelial cells, enhances their permeability and destroys pre-existing vasculature. Remarkably, all of this can be achieved only with nanomolar TH-482 concentrations. At the same time, micromolar concentrations of TH-482 are required for inhibition of microtubule polymerization. These findings are no different from other MTAs such as paclitaxel, epothilones, CA-4 sulfonate analogs, T138067 and 2-

(3,4,5-trimethoxybenzoyl)-3-amino 5-aryl thiophenes.[132]

Microtubule-stabilizing agents in pancreatic cancer
Paclitaxel

Taxanes is a novel class of anti-tumor drugs extracted from the stem bark of *Taxus brevifolia*, a Pacific yew tree found in the western United States, in 1966. The structure of paclitaxel was discovered in 1971, but its microtubule-stabilizing characteristics were identified only 8 years later, in 1979.[149] Paclitaxel is one of the most effective microtubule-targeting anti-cancer drugs. Paclitaxel was approved by the FDA in 1992, and is still considered to be one of the most critical supplements to chemotherapeutic regiments against various cancers. Notably, it was approved by the FDA for the first-line treatment of PC in 2012.[144] At present, paclitaxel combined with albumin-based chemotherapy is used as the first-line of advanced PC therapy. Paclitaxel influences the dynamics and microtubule polymerization via binding to taxane site, which leads to cell cycle arrest and cell death. Because paclitaxel dramatically decreases cell proliferation and mitotic rate of microtubules at lowest concentrations without significantly rising polymer levels, suppression dynamics of microtubule appears to be its most effective mechanism of mitotic arrest. Paclitaxel at high concentrations promotes addition of tubulin dimers and disturbances in dynamic balance of microtubules, but acts the opposite at low concentrations.[115] Several approaches have been implemented to improve solubility and pharmacology of paclitaxel such as albumin nanoparticles, liposome and emulsions.[144] Albumin-stabilised nanoparticle formulation of paclitaxel is also known as ABI 007, or nab-paclitaxel.

Tumors collected from untreated animals group were stained with Collagen IV and Masson's trichrome that revealed enormous levels of fibrotic tissue in the tumor microenvironment. The visually impressive decrease in fibrotic tissue mass was noted in tumor tissues after administration of nab-paclitaxel compared with those treated with paclitaxel. Nab-paclitaxel therapy decreased the amount of proliferating carcinoma cells to a greater extent than paclitaxel therapy as evidenced by a decreased amount of carcinoma cells expressing Ki-67. Nab-paclitaxel plus gemcitabine therapy was very useful in decreasing Ki-67 (+) tumor cells compared with paclitaxel plus gemcitabine treatment. Plasma and intratumor concentrations of paclitaxel following nab-paclitaxel or paclitaxel therapy were performed to investigate the potential mechanism of the therapeutic effectiveness of nab-paclitaxel over paclitaxel. Nab-paclitaxel therapy was correlated with higher tumor stroma in the tumor microenvironment compared with paclitaxel-treated and untreated tumors.

According to results from both clinical and preclinical studies, efficacy of nab-paclitaxel is superior to that of cremophor-based paclitaxel owing this to many factors including better pharmacokinetics behavior. PDAC cells represent macropinocytosis and albumin internalization through macropinocytosis are implicated in PDAC. Additionally, a higher intratumor paclitaxel concentration was achieved after nab-paclitaxel treatment, resulting in the desmoplastic tumor stroma destruction and enhanced neoplastic cells death. This may be another reason of superiority of nab-paclitaxel over paclitaxel treatment in PDAC.[122]

Nab-paclitaxel

Nab-paclitaxel (Abraxane®) is a 130-nm, solvent-free, albumin-bound formulation of paclitaxel. Apart from hindering cell division via interrupting microtubule network, it can enhance transportation of paclitaxel to endothelial and tumor cells. Nab-paclitaxel has many advantages compared with sb-paclitaxel. For instance, it produces significantly higher doses of paclitaxel in a shorter transfusion time (30 minutes vs 3 hours for sb-paclitaxel), can reach a higher peak concentration, enhance drug combination to tumors and endothelial cells more effectively. Another study showed the nab-paclitaxel had a higher prompt neoplasm uptake comparing to sb-paclitaxel after administration at equal doses.

In phases I and II trials, a maximum-tolerated dose of nab-paclitaxel and gemcitabine (1000 mg/m^2 and 125 mg/m^2, respectively) were given to advanced PDAC patients (QW 3/4 w). 44 patients that received this treatment had overall response rate of 48% and the median overall survival of 12.2 months. In Phase III trial, 850 patients with metastatic PDAC receiving the same regimen were compared with monotherapy of gemcitabine 1000 mg/m^2 (QW 7/8 w as cycle 1 and QW 3/4 w as cycle 2). Median overall survival was significantly longer in nab-paclitaxel plus gemcitabine group (8.5 vs 6.7 months). Other trials mentioned that the grade 3 neuropathy was correlated with nab-paclitaxel in a majority of patients with advanced PC, and improving to grade 1 took a median of 29 days.[117]

Effects of gemcitabine, nab-paclitaxel and no treattment were investigated in the following PDAC cell-lines: MIA PaCa-2, AsPC-1, BxPC-3 and Panc-1a. Addition of nab-paclitaxel or docetaxel at IC25 reduced IC50 of gemcitabine. Tumor growth inhibition after gemcitabine, nab-paclitaxel and docetaxel was 67%, 72% and 31%, respectively. Tumor stromal mass, estimated through the reduction in α-smooth muscle actin, collagen I and S100A4 expression, was greater reduced by nab-paclitaxel than docetaxel. Furthermore, PDAC xenograft model study showed that nab-paclitaxel is more efficacious and results in a longer median survival than gemcitabine. Phase I, II and III trials were performed to examine nab-paclitaxel-based chemotherapy together with target therapy or immunotherapy in metastatic PDAC patients.[118] Nab-paclitaxel plus gemcitabine therapy comprises standards of metastatic PC care, and this combination is suitable for PDAC patients with different characteristics and clinical presentations.[119]

Secreted protein acidic and rich in cysteine (SPARC) has a crucial role in the transport of nab-paclitaxel to tumor. A research was conducted to examine the relationship between prognosis of patients receiving nab-paclitaxel plus gemcitabine plus and SPARC expression.[117] In phases I and II, stromal SPARC expression (high and low) was significantly associated with OS in the nab-paclitaxel plus gemcitabine group (17.8 vs 8.1 months), indicating that SPARC may serve as a biomarker for PC. However, phase III concluded that intratumor, stromal and plasma SPARC were not predictive of survival rate in either groups with metastatic PC.

As technologies advance, nab-paclitaxel undergoes additional inquiries in PDAC therapy. The solvent-free albumin-paclitaxel nanoparticles are comparatively more favorable than solvent-based formulations of cre-paclitaxel in patients with advanced metastatic PC. Quitting the treatment with albumin-paclitaxel is associated with a lower risk of neutropenia, infusion hypersensitivity responses and quicker alleviation of external neuropathy. Albumin-paclitaxel is currently regarded as an ideal regimen for patients with metastatic PDAC. Albumin-bound formulation reduces tumor stroma via synergy between albumin and SPARC, thereby affecting tumor microenvironment. This mechanism promotes gemcitabine-enhanced effect.

Several studies examined the efficacy and survival advantage of nab-paclitaxel alone and in combination with gemcitabine. They aimed to study treatment effect on tumor cell proliferation, tumor desmoplasia and metastases to adjacent organs.[121] Nab-paclitaxel as an individual agent was not found to be significantly useful in decreasing primary tumor weight or increasing mouse survival rate compared with nab-paclitaxel or gemcitabine monotherapy. Finally, combined treatment of gemcitabine and nab-paclitaxel reduced metastatic tumor burden and elevated median survival rate of animals greater than any of the agents alone.[120] The synergy between nab-paclitaxel and gemcitabine in PDAC was assessed in two preclinical models: genetically engineered mice and primary patient-derived tumors. The result of the experiment in the primary tumor xenograft model demonstrated that nab-paclitaxel plus gemcitabine induced regression of tumor in 64% of the 11 biologically different primary tumors versus 36% and 18% nab-paclitaxel and gemcitabine monotherapy, respectively. Another study's outcomes showed that nab-paclitaxel treatment was more effective in preventing initial tumor progression, solid tumor stroma depletion, consistently showing a higher anti-tumor response and increased survival rate in animal models than paclitaxel treatment. Combined treatment of gemcitabine plus nab-paclitaxel reduced metastatic tumor burden and improved overall survival rate of animals compared with monotherapy of any of the agents. Moreover, it was found that there is no benefit of adding paclitaxel to gemcitabine treatment for regionally advanced and metastatic PDAC.[115] In 2013, gemcitabine plus nab-paclitaxel was approved by the FDA as the first-line treatment for patients with metastatic PC. Nab-paclitaxel plus gemcitabine was found to be the best combination to improve tumor response and survival rates in metastatic PDAC compared with gemcitabine alone.[116]

Treatment with nab-paclitaxel seemed to exhaust the desmoplastic stromal matrix and improve microvasculature in gemcitabine-resistant primary tumors. Intratumoral gemcitabine concentration was 2.8 times higher in mice receiving nab-paclitaxel plus gemcitabine than gemcitabine alone. Related synergistic anti-tumor and pharmacologic responses were confirmed in a transgenic PDAC murine model. It showed that paclitaxel elevated intratumoral accumulation of gemcitabine via inactivation of cytidine deaminase, an enzyme that inhibits gemcitabine. Another study revealed that nab-paclitaxel plus gemcitabine therapy efficiently reduced the density of tumor-associated fibroblasts and produced disruptive changes in tumor stroma. Preclinical trial results revealed the positive anti-tumor activity of nab-paclitaxel and its potential to alter desmoplastic stroma. This was the part of MPACT trial, a randomized phase III study, which confirmed efficacy of nab-paclitaxel plus gemcitabine. Positive conclusions from this research have directed to the regulatory approval of this therapy, which is currently being performed as a standard of care regimen in the therapy of patients with advanced metastatic PDAC.[120]

Epothilones

Epothilones are a novel class of anti-microtubule agents derived from the soil bacterium *Sorangium cellulose*. They bind to taxane site and stabilize microtubules polymerization. Epothilones have the activity of promoting assembly and polymerization of microtubules. After binding to the microtubule, epothilones restructure the disordered M-loop (site of lateral tubulin contacts) within microtubule and thus stabilize microtubules. Compared with paclitaxel, epothilones have following advantages. First of all, the activity of epothilones is 10 to 1000 times higher than that of paclitaxel. Epothilones display higher bond and strength compared with the paclitaxel. Secondly, the water solubility is also higher. Lastly, the structure is much simpler making them easier to synthesize.[123] Thanks to these advantages, epothilones were introduced to Oncologist's portfolio of drugs, which represents a pivotal step in PDAC therapy.

Human PC xenograft model experiment demonstrated effectiveness of one of the derivatives of epothilone B against PDAC. This derivative is named ixabepilone, previously known as BMS-247550. Ixabepilone is more efficient in inhibiting tumor growth than paclitaxel with 5–6 lower doses required in mice and rats. Phase II trial (Southwest Oncology Group) suggests that ixabepilone is efficacious in the treatment of patients with PC. The treatment with this compound in patients with metastatic PC resulted in median survival of 7.2 months and 6-month survival of 60%, whereas the median survival of

gemcitabine was 5.65 months and 6-month survival of 46%.[124]

(Z)-1-(2-bromo-3,4,5-trimethoxyphenyl)-3-(3-hydroxy-4-methoxyphenylamino)-prop-2-en-1-one (10ae)

Small synthetic molecules (10ae) molecules promote tubulin polymerization and induce apoptotic death of PDAC cell lines (MIA-Paca2 and Panc-1 cell lines).[150]10ae behaves like paclitaxel and epothilones in cancer cell killing *in vitro*, which is stabilizing microtubules.

10ae has a remarkable cell killing ability. It induces apoptosis in 20 tumor cell lines with similar GI50 values. Such broad spectrum of action is probably due to its inhibitory effect on critical stages of cancer cell division. Flow cytometry concluded that 10ae arrested the cells in the G2/M phase and that it may trigger apoptosis through activation of caspases, which was assessed by poly(ADP-ribose) polymerase-1 (PARP) cleavage.

Treatment with 10ae leads to tumor cells accumulation in the G2/M phase in a dose-dependent manner. They begin to accumulate in the G2/M phase already after administration of 0.25 μM of 10ae and present with G2/M arrest after treatment with twice the dose. The fluorescence-activated cell sorting showed that the treated tumor cells had >2N DNA regardless of whether it was G2 or M phase. Influence of 10ae on the phosphorylation of proteins serving as markers of SAC activation and mitotic arrest (history H3, Bcl-2, BubR1) was then studied. All 3 were phosphorylated 6 hours after 10ae treatment showing similar increase in concentration level (2.95-fold). To conclude it all, 10ae has the most potent cytotoxic properties among other derivatives of 10, and it surpasses paclitaxel in a way that it causes more microtubule assembly at the same concentration (1 μM).[112]

Mechanism of microtubule-targeting drugs in cancer therapy

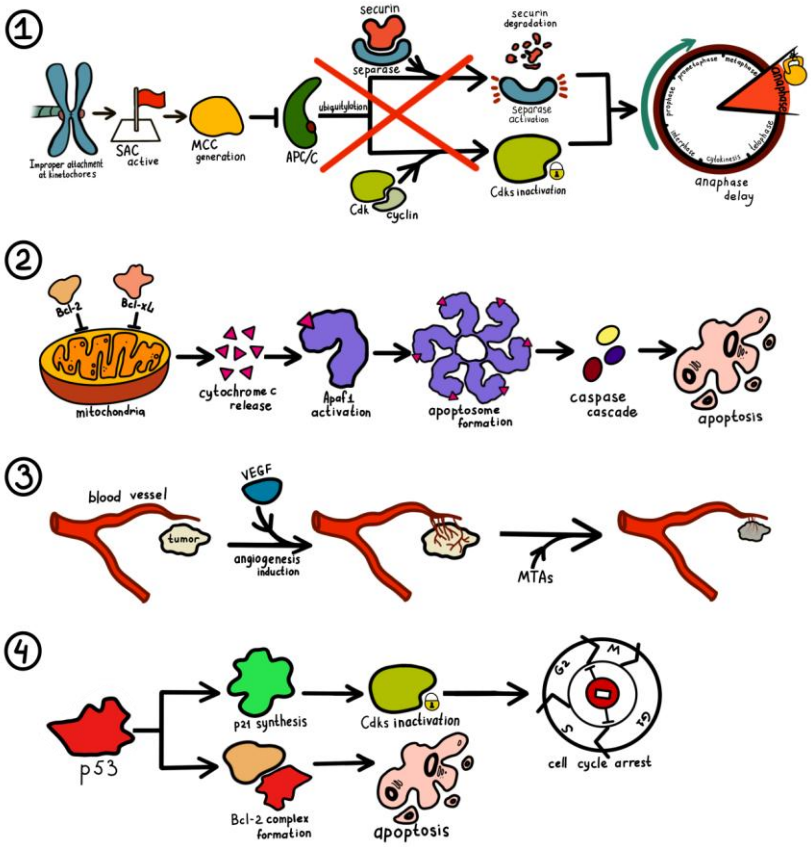

Fig. 4: Mechanism of microtubule-targeting drugs in cancer therapy. 1. Improper, incomplete or absent attachment at kinetochores maintain spindle assembly checkpoint (SAC) activity. When it is "on", a group of checkpoint proteins constituting mitotic checkpoint complex (MCC) is recruited and block the activity of anaphase-promoting complex/cyclosome (APC/C). As ubiquitylation of cyclins and securin does not take place, a cell's entry into anaphase is impossible. 2. MTAs trigger phosphorylation of Bcl-2 and Bcl-xL, allowing for cytochrome c release by mitochondria. Produced cytochrome c bind to apoptosis-protease activating factor 1 (Apaf1) and result in generation of apoptosome. Eventually, caspase cascade is triggered leading to apoptosis. 3. Tumor cells increase endothelial cell proliferation and vasopermeability and alter gene expression via vascular endothelial growth factor (VEGF) pathway. Ensuing angiogenesis facilitates tumor cell proliferation. MTAs cut off tumor blood supply by destroying its vasculature. 4. Increase in p53 concentration stimulates production of p27 that inhibits cyclin-dependent kinase (Cdks) and thus prevents cell cycle transition at several checkpoints. P53 can also interact with some members of Bcl-2 family and induce apoptosis via aforementioned mechanism.

1. Block cell mitosis and cycle progression

When bound to the specific site of tubulin molecule, MTAs alter normal structure and function of microtubule. This affects microtubule assembly and spindles formation. Mitotic spindles lose pulling power requited to separate sister chromatids and cannot properly orientate them, which completely stops the process of cell division following metaphase. When the process of cell division is affected, mitotic checkpoints receive a response and block the cell cycle. The two-way separation of sister chromatids is regulated by SAC. Chromosome segregation will not occur until the "correct" checkpoint is determined, which is defined as a proper and stable kinetochore-microtubule attachment.[133] To ensure that anaphase will not start when kinetochores are not attached or attached improperly, checkpoint proteins are recruited on kinetochore forming a mitotic checkpoint complex (MCC). This complex inhibits anaphase-promoting complex/cyclosome (APC/C) preventing degradation of securin and cyclin proteins and thus anaphase onset. Errors in the test point of the cell cycle of tumor cells may result in drug sensitivity differences due to changes in the structure or expression of test point kinase.[134]

As mentioned earlier, MTAs induce cell cycle arrest in the G2/M phase. Paclitaxel reserves to two mechanisms to block the cell cycle: it can regulate the expression of cyclin B1, and cyclin-dependent kinase (Cdk). CA-4 blocks the cell cycle in the G2/M phase by regulating the expression level of Cdc2.[135]

2. Induce apoptosis and autophagy

Cell cycle arrest has long been known to trigger apoptosis, which is a common mechanism of action of MTAs. Apoptosis induction occurs via different pathways, such as phosphorylation of Bcl-2 and Bcl-xL, activation and upregulation of E2F1 - all of which can instigate the release of cytochrome c.[50] Activation of mammalian target of rapamycin (mTOR) is also implicated with microtubules. Interference with activity of microtubules by MTAs interrupts the AKT/mTOR signaling pathway leading to hindered tumor cell proliferation via autophagy induction. This independent mechanism represents a unique tool for inducing mitotic arrest.[136]

3. Anti-angiogenesis and vascular destruction

Folkman et al. postulated that tumor neovascularization is involved in tumor development and metastases.[151] Destruction of tumor vasculature, starvation of tumor cells and other strategies have been widely adopted in many types of cancer. Several cancers in mice were found to be inhibited after feeding with natural vascular inhibitors.[134]

Application of MTAs becomes a new direction in the research of anti-tumor drugs. Drugs including paclitaxel, vinblastine and colchicine have potential anti-angiogenic effects on PC. Vinblastine exhibited dose-dependent anti-angiogenic activity in the chick embryo chorioallantoic membrane model. The researches confirmed that tumor blood vessels could be selectively destroyed within 6 hours after administration of CA-4 in alive rat model. Notably, effect on the intratumor blood vessels is stronger than those of the extratumor, and significant effect on the normal vasculature was observed. Some researchers are currently investigating the effect of MTAs on vascular destruction and conducting corresponding clinical trials. More than ten kinds of tumor vasculature-targeting drugs are enrolled in clinical trials. Most of them act on colchicine site in PC and advanced solid tumors. CA-4 stands out as a vascular destructor among MTAs. It has successfully passed phases I and II clinical trial.

Only a few studies have observed the side effects of MTAs such as cardiotoxicity and gastrointestinal adverse reactions. Also, it is still unclear as to how MTAs exactly inhibit tumor angiogenesis or destroy blood vessels feeding tumor. Vascularization requires proliferation and transport of vascular endothelial cells, both of which are very sensitive to MTAs. Certain MTAs are speculated to affect the development of tumor blood vessels by altering the expression of vascular endothelial growth factor (VEGF). Colchicine, nocodazole, vinblastine and vincristine were shown to reduce production of human umbilical vein endothelial cells (HUVEC) and expression of VEGF.[78,137]

Finally, damage to tumor vasculature inflicted by MTAs is more reversible than inhibiting cell proliferation. Drugs that cause depolymerization for a short period of time are more suitable to serve as vascular inhibitors, whereas long-term anti-mitotic agents would act as tumor cell proliferation inhibitors.[138]

4. p53

The core mechanism of paclitaxel affecting mitosis of cancer cells is still under investigation. Some studies proved that the inhibitory action of paclitaxel lies in alterations of microtubule transport that lead to halted activation and translocation of androgen receptors decreasing tumor proliferation. In addition, paclitaxel can stimulate production of tumor suppressor protein, p53, and increase its quantity in the nucleus.[139,152] The loss of p53 function due to defective genome unleashes build-up of tumorigenic mutations in the cell and increases cancer cell survival. p53 signaling is associated with microtubule dynamics and expression of various tubulin isotypes.[50]

CONCLUSION

Tubulin is not the most optimal target for cancer targeting drugs as it requires high selectivity of agents. This in turn makes drug developing rather complex. However, current researches give hope that creation of such agents is possible and prolonging survival rate in PC may not be unachievable. Humanity has long needed it.

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CONFLICT OF INTEREST
The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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ABBREVIATIONS
PDAC, pancreatic ductal adenocarcinoma; PC, pancreatic cancer; GTP, guanosine triphosphate; GDP, guanosine diphosphate; MAP, microtubule-associated protein; HPDE, human pancreatic cell lines; MTAs, microtubule-targeting agents; MDAs, microtubule-destabilizing agents; MSAs, microtubule-stabilizing agents; NSCLC, non-small cell lung carcinoma; 2ME, 2methoxyestradiol; PDT, podophyllotoxin; PLA, Peloruside A; LAU, Laulimalide; CA-4, Combretastatin-A4; SPARC, secreted protein acidic and rich in cysteine; SAC, spindle assembly checkpoint; MCC, mitotic checkpoint complex; APC/C, anaphase-promoting complex/cyclosome; Cdks, cyclin-dependent kinases; mTOR, mammalian target of rapamycin; VEGF, vascular endothelial growth factor.

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