

Tubulin Role in Cancer Development and Treatment

Vsevolod Dolhyi, Dmytro Avierin and Mohammad Hojouj*

Department of Oncology and Medical Radiology, Dnepropetrovsk Medical Academy, Dnepropetrovsk, 49102, Ukraine

***Corresponding author:** Hojouj Mohammad, Department of Oncology and Medical Radiology, Dnepropetrovsk Medical Academy, 31, Blyzhnya Street, Dnepropetrovsk, 49102, Ukraine, Tel: +380567560399/ +380675673277; Fax: 380563777797; E-mail: hojouj@yahoo.com

Abstract

This review work is done to show a significance of tubulin in cancer development. Within last decades there are a lot of studies have performed in this area. Now it is clear that there are an enormous number of functions in cell performing by microtubules, a structure unit of which is tubulin. Now it used widely as a predictive factor of tumor aggressiveness, but increasingly it becomes a target for studying and treatment elaboration, since it is well-known that to now a day's tubulin-targeted medicines, such as taxanes or vinca-alkaloids, resistance develops rather quickly, so it consists a large problem in oncology. This work reveals basic microtubule functions, violations that it may undergo and consequences of these. Also it is described here the main modern tendencies in creation of remedy which will make it possible breakthrough treatment resistance barrier.

Keywords: *Tubulin; Cancer development; cancer treatment*

Received Date: July 27, 2019; **Accepted Date:** August 31, 2019; **Published Date:** September 07, 2019

Introduction

Cancer or malignant neoplasm is a genetic disorder that results from genetic or epigenetic alterations in the somatic cells. It's well-known fact that cancer development is multistep process. Genetic and epigenetic changes are involved in alterations which ultimately lead to malignant transformation of the healthy cells [1].

Modern direction of cancer treatment strives to targeted and personalized therapy, but unfortunately such expensive methods turn out low effective [2].

This fact cause necessity to search different ways to resolve this problem not only by scientist but also pharmaceutical companies, which are interested the most to invest in really acting approaches and medicines.

Patients with stage III or IV diseases who are treated in clinics, often advance to metastatic stages and develop drug resistance and relapse involving lymph nodes, liver, lungs, bones, and brain resulting in systemic multiple organ failures (MOFs) [2].

Especially interesting as have been noticed in a lot of analyses of tumors of diverse localizations that it very often correlates with high tubulin expression, more precisely its TUBB3 isotype. One of such investigations has proved that enhanced TUBB3

Citation: Hojouj Mohammad, Tubulin Role in Cancer Development and Treatment. *Cancer Med J* 2(2): 45-54.

expression is significantly associated with an advanced depth of tumor infiltration ($p < 0.003$) and the existence of lymph node metastases ($p = 0.023$). The 5-year survival rate of the whole cohort in this survey was 35% and the median overall survival 2.15 (95% CI = 1.86-2.67) years. The TUBB3 protein high expressing patients demonstrated a significantly ($p = 0.0003$) worse prognosis with 16% 5-year survival rate and a median overall survival of 1.27 (95% CI=1.05-1.92) years compared to the low expressing patients (5 year survival 46%, median overall survival 382 years). It was determined that high protein and mRNA expression levels of TUBB3 (class III β -tubulin) are associated with aggressive tumor features in esophageal adenocarcinomas [3]. Such results suggest that TUBB3 expression level can be reasonably accurate predictive factor.

Issues connected with tubulin consist a huge problem for all scientists and physicians which are looking for solutions to this problem. It is well-known that high-aggressive tumors initially often respond well to treatment, but later returns being far more aggressive than before, resulting in multi resistance and as consequence leads to death. It raises some of the main issues in oncology: which alterations lead to such aggressive behavior when relapse, and how can we counter this (Figure 1).

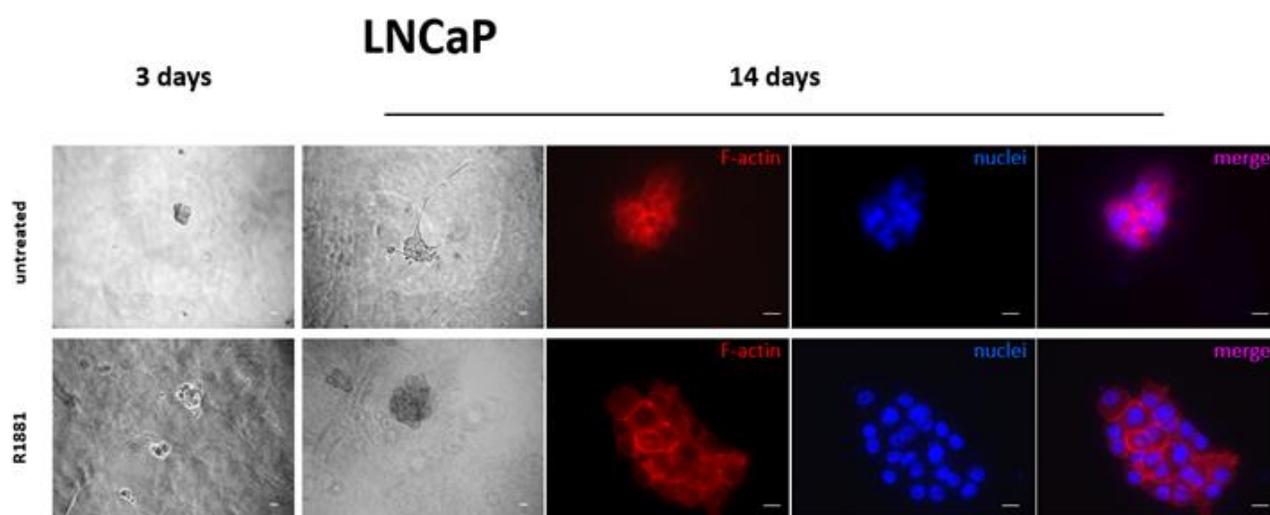


Figure 1: Tubulin immunofluorescence. Changes in the quantity and quality of tubulin and him subspecies (cytoskeleton) in prostate cancer cells (LNCaP). On top: the cells were not stimulated by androgens. Bottom: The cells were stimulated by androgens for 14 days.

One solution came from studying of protein which plays one of the main roles in cell behavior - tubulin, which basically exists as microtubules structural unit.

Microtubules are cylindrical polymers composed of α - and β -tubulin subunits. These subunits associate longitudinally to form protofilaments and the protofilaments associate laterally to generate a microtubule [4].

Microtubules are highly dynamic polymers as their ends can undergo rapid lengthening and shortening by the addition and removal of tubulin heterodimers, a phenomenon termed 'dynamic instability' [5].

They are constantly lengthening and shortening throughout all phases of the cell cycle. During interphase, microtubules are nucleated at the centrosome (minus end) and radiate toward the cell periphery (plus end). Interphase microtubules are involved in the maintenance of cell shape and in the trafficking of proteins and organelles. Motor proteins translocate cell components

on microtubule tracks, and protein–protein interactions with other adaptor proteins co-ordinate this process. Tubulin heterodimers also exist in soluble form in cells, and protein interactions with this tubulin population regulate microtubule behavior. The addition and removal of soluble tubulin heterodimers to dynamic microtubule ends is a highly regulated process [6].

During mitosis microtubules form the spindle to enable correct chromosomal segregation. Tubulin-binding agents (TBAs; e.g., taxanes, vinca alkaloids, epothilones, and eribulin) are important chemotherapeutic drugs that suppress spindle dynamics, causing subsequent mitotic arrest and cell death in rapidly dividing cells [10].

In humans, microtubules are composed of combinations of nine α -tubulin isotypes and nine β -tubulin isotypes, with the different tubulin isotypes possessing specific tissue and developmental distributions. The members of the tubulin family share a high degree of structural homology and are distinguished from one another by highly divergent sequences at their carboxy-terminal (C-terminal) tail [6].

Main Microtubules Functions Overview

Basic functions which microtubules perform in cell have become targets for investigating and further elaboration of medicines. So it is necessary to review shortly this ones.

Microtubule cytoskeleton in stress responses

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

Microtubules and cellular signaling

While microtubules possess distinct functions in particular stress responses, the microtubule network also influences common signaling pathways engaged by a variety of cellular stresses

Microtubules and p53

p53 is a key mediator of cellular stress responses and its activity heavily depends on microtubules. p53 is translocated to the nucleus along microtubule tracks by dynein proteins in a complex with heat shock protein 90 (Hsp90) and Hsp90 immunophilins.

Hypoxia

In the absence of oxygen, HIF1 α heterodimerize to the constitutively active β subunit to initiate transcriptional changes.

Oxidative stress

Disturbed oxidative stress signaling has been determined as one of the widespread mechanisms of cancer development. Tubulins interact with mediators of the oxidative stress response, with direct interactions between β III-tubulin and glutathione S-transferase μ 4 observed in ovarian cancer cells [6].

Metabolic stress

The main reason why metabolic stress occurs in cancer cells is its uncontrolled division, resulting in disparity between nutrients needs and its supplying. Microtubules and tubulins are involved in responding to metabolic stress by sensing and modulating metabolic processes to maintain cellular energy levels. The microtubule network is hypothesized to play a critical role in the regulation of cellular metabolism [11].

Metabolic regulation

Tubulins and microtubules have been suspected to function as a key modulator of mitochondrial metabolism for some time. Recent studies have demonstrated that tubulin is capable of interacting with, and blocking the VDAC, thereby regulating ATP and metabolite compartmentalization and contributing to the Warburg effect.

Autophagy

Macroautophagy (hereafter referred to as autophagy) can be induced in cells in response to diverse stresses, including metabolic and ER (endoplasmic reticulum) stress. Microtubules have been known to play a critical role in autophagic flux.

Protein folding stress

Misfolded proteins may arise from protein damage, inadequate chaperone activity, and malfunction of protein processing systems. Endoplasmic reticulum is highly important organelle for correct folding of membranous and secretory proteins, and it very depends on current cell condition, so such serious changes as malignancy significantly disturb function of ER.

Mitochondrial function

As integrators of cell state and mediators of apoptotic signaling, mitochondria play a critical role in determining cell fate in response to stress. There is growing evidence that tubulin, microtubules, and the microtubule network regulate mitochondrial function in cancer. Microtubules exactly that structures which influence mitochondria trafficking and degradation, thereby sustaining its own stability and function on certain level.

Cell death signaling

It's known that failure of cellular cell responses can result in cell death induction. Emerging evidence supports a role for tubulins and microtubules in the execution of cell death in response to stress. For instance, tubulins interact with regulators of mitochondrial membrane permeability and apoptosis [6].

Centrosomes and cell division

Centrosomes are the major microtubule organizing centers (MTOC) of mammalian cells. Each centrosome consists of a pair of centrioles surrounded by pericentriolar material (PCM), from which spindle and astral microtubules emanate. In healthy cells, strict regulation of centrosome duplication ensures the formation of only two functional centrosomes, which assemble bipolar spindles to avoid chromosomal aberrations in mitosis. In contrast, many cancer cells harbor extra centrosomes accompanied by chromosomal instability. Thus, centrosome amplification and its structural aberrations is a hallmark of human cancers and has direct consequences on chromosomal instability and cancer cell invasion [1].

Tubulin and VDAC

Last time there have been appeared a lot of reports about cytoskeleton dependence on bioenergetic function of cell. The main player in these models is the voltage-dependent anion channel (VDAC), located in the mitochondrial outer membrane. Most metabolites including respiratory substrates, ADP, and Pi enter mitochondria only VDAC. It's has been established that passing of such high-energy substrates trough VDAC is regulated by β -tubulin, which bound to it. So it is presumed that such β -tubulin-VDAC interaction participates in regulation of cancer metabolism, being an important factor switching from oxidative phosphorylation, usually occurring in normal cells, to glycolysis, which is a distinctive feature of cancer cells [7].

Microtubules are responsible for the mitochondrial subcellular arrangement and dynamics in skeletal and heart muscle cells. Their cytoarchitecture, isoform composition, interaction partners, and post-translational modifications act as spatial cues to navigate mitochondria within the cellular space and help to stall and anchor mitochondria at sites of high energy demand [8].

Events that violate microtubules activity

The clinical observations are supported by numerous in vitro studies where altered β III-tubulin levels confer resistance to a broad spectrum of drug classes in solid and hematological tumors [9].

Growing evidences about significance of β III-tubulin in tumors development and aggressiveness suggest that it is one of the main survival factors in cancer, consequently it has become an object of close attention among cancer researchers [6]. So what should happen to microtubules, largely TUBB3 isotype, it to show such altered behavior and high-aggressive course of disease. There are at least nine isoforms of α -tubulin and nine isoforms of β -tubulin in humans [12].

Changes in tubulin composition

Every tissue in human body has specific ratio of different tubulin isoforms, resulting in countless quantity of combinations. Features of its functioning is still poorly understood. But one for sure, that these specific combinations reflect qualities of metabolism in every particular tissue. Resulting from it we can conclude that changes in its number or ratio may be one of possible mechanisms of cancer progression.

Altered tubulin isotype expression is the most widely characterized microtubule alteration reported in cancer and has been observed in both solid and hematological tumors. High β III-tubulin level correlates with much poorer prognosis for patients with epithelial cancer. But it's interesting that it associates not only with TBA resistance, but also β III-tubulin influence sensitivity to non-tubulin targeted agents. The clinical observations are supported by numerous in vitro studies where altered β III-tubulin levels confer resistance to a broad spectrum of drug classes in solid and hematological tumors [6].

All these appeared proofs gave possibility to look another at tubulin role in cancer and features of treatment choosing depending on this factor [13].

Tubulin post-translational modifications

As it is known microtubules are highly conserved structures and PTM is a good explanation of diverse functions carried by microtubules. Tubulins are subject to diverse post-translational modifications (PTMs). There are numerous of PTMs whose exact influence on tubulin remains poorly understood. Post-translational modifications are thought to regulate protein-protein interactions with the microtubule cytoskeleton, thereby affecting signaling events within the cell. The majority of these

modifications are localized to the tubulin C-terminus and potentially impart specific functions to the different tubulin isotypes. Microtubules and their role in cellular stress in cancer [6].

For example, acetylated tubulin is implicated in intracellular trafficking, endoplasmic reticulum (ER) localization, and ER-mitochondria interactions, as well as the regulation of microtubule dynamics [14].

Removal of the final two residues of the β IVb-tubulin C-terminal tail was identified in higher stage liver cancer and in a mouse model of hepatic carcinoma [15].

Microtubule-associated proteins

A wide variety of proteins are known to interact with tubulins. One more factor which interacting with tubulin is responsible for its stability and treatment resistance is microtubule-associated proteins (MAPs). Aberrant expression of primarily neuronal MAPs (e.g., Tau, MAP2) has been detected in non-neuronal cancer tissue [6].

For example, it has been determined that one of such MAPs is tau, high level of which associates with poor prognosis in breast cancer and decreased sensitivity to taxane due to change of drug affinity to β -tubulin [16].

Tubulin localization in cell nuclei

One study has established that β II-tubulin also is found in cell nuclei, moreover it can exist in non-microtubule form and significantly impact on cell behavior. β II-tubulin may facilitate cancer growth and metastasis and, to accomplish this, may not need to be in microtubule form [17].

Specific nuclear localization of β II-tubulin was demonstrated not only by immunohistochemistry using a monoclonal antibody to β II, but also by immunoblotting of a purified nuclear fraction, and by the fact that fluorescently labeled $\alpha\beta$ II-tubulin when micro-injected into these cells, went into the nuclei whereas fluorescently labeled, micro-injected $\alpha\beta$ III and $\alpha\beta$ IV did not [18].

It was found that over-expression of β II was correlated with a shorter life expectancy of patients with CRC. The life expectancy was even shorter for patients in whose tumors β II was localized to the cell nuclei [17].

Current directions in solution of tubulin questions

Appearance of quiet detailed knowledge about tubulin and its functions within last decades led to elaboration of different ways to breakthrough this checkpoint in cancer study and create universal remedy, which provides to higher survival rate of oncology patients with diverse localization of tumors.

Inhibition of CPAP - tubulin interaction

CPAP - is a centrosome-centrosomal protein associated protein. Centrosome amplification is a distinctive trait of cancer which play an important role in invasion. To survive, cancer cells cluster amplified extra centrosomes and achieve pseudo bipolar division. It became a cause to elaborate ways to prevent clustering of extra centrosomes. Tubulin, by interacting with the centrosomal protein CPAP, negatively regulates CPAP-dependent peri-centriolar material recruitment, and concurrently microtubule nucleation. Screening for compounds that perturb CPAP - tubulin interaction led to the identification of CCB02 (a selective inhibitor of CPAP-tubulin interaction), which selectively binds at the CPAP binding site of tubulin. Genetic and chemical violation of CPAP - tubulin interaction causes extra centrosomes to nucleate enhanced numbers of microtubules prior

to mitosis. This causes cells to undergo centrosome declustering, prolonged multipolar mitosis, and cell death. Assay in which was researched CCB02 reveals high anti-invasive activity in different cancer types, in particular in tyrosine kinase inhibitor (TKI)-resistant EGFR-mutant non-small-cell lung cancers. So, it is a perspective direction to use centrosome quantity as marker to develop global new treatment approach, especially those which exhibit high incidence of centrosome amplification [1].

β III/ β IV-tubulin inhibitor (VERU-111) is new developing remedy for pancreatic cancer

Recent studies have reported major roles of β III and β IV-tubulins in pancreatic cancer as these isotypes are highly expressed in pancreatic tumors, while absent in normal pancreas (acinar and pancreatic islets) [19].

The expression of these tubulins has been associated with pancreatic cancer progression, metastasis and chemoresistance. Additionally, β III-tubulin knockdown reduced the pancreatic tumor growth and metastasis in an orthotopic xenograft mouse model. A novel synthetic molecule that is known to overcome multidrug resistance preferentially inhibits the expression of β III and β IV-tubulins via restoring the expression of miR-200c in pANCA cells. Also it was demonstrated that VERU-111 effectively inhibits growth and metastatic phenotypes of pancreatic cancer cells in in vitro and in vivo model systems [20].

Developments of Chinese researchers - Antiproliferative aspidosperma-type monoterpenoid indole alkaloids from *Bousignonia mekongensis*

Innate and acquired drug resistance, especially multidrug resistance (MDR), are major obstacles in cancer chemotherapy. In this survey was determined that high level of P-glycoprotein correlates with poor prognosis, at the same time the most anti-microtubule drugs such as paclitaxel or vincristine are its substrates. Recent study established that there is another, the colchicine site (CS) on the α/β tubulin dimer, antimitotic agents to which turned out highly effective in cells with β III-tubulin overexpression. So, a new approach to treatment with anti-tubulin agents has appeared, and what is important it can break through resistance to general used anti-tubulin drugs targeted to P-gp.

In order to find such colchicine site substrates investigators sought among monoterpenoid indole alkaloids which are structurally diverse natural products found in plants of the family Apocynaceae. Among them, vincristine and its derivatives are well known for their antiproliferative activity. Among them, vincristine and its derivatives are well known for their anticancer activity. There are a lot of different monoterpenoid indole alkaloids in this species. To be more precise there are fourteen alkaloids, which were isolated and investigated, consequently got numbers from 1 to 14. It was determined that alkaloids 3,6,9,13 have similar spectra of action and turned out effective even in multi-resistant subline cells. The mechanism is in cell cycle arrest at the G2/M phase by inhibiting tubulin polymerization as well as mitotic bipolar spindle formation. This analysis also suggested that a 14,15-double bond or 3 α -acetyl group is critical for enhanced antiproliferative activity [21].

Investigation of benefit from tubulin-targeted and immune therapeutics

Immune therapy of cancer recently has become very promising direction of treatment of many cancer types. Appeared results that tubulin is close connected with immune response has increased interest of combining immune and tubulin-targeted drugs. There are two main processes occurring among microtubules - polymerization and depolymerization. So, anti-tubulin drugs also divide into two groups: anti-depolymerization agents such as the taxane family, and anti-polymerization agents such as colchicine and vinca alkaloids. They interact with immune system in different ways even within the same class. It was determined that certain anti-polymerization agents such as colchicine appear to depress most immune cell types, while inducing dendritic cell maturation and increasing M1 macrophage population. In contrast, the vinblastine anti-polymerization agent

activates many of these cell types, although down regulating Treg cells. The combination of tubulin-targeting anticancer agents and immune therapy appears to be especially promising, as several lines of evidence suggest that agents functioning as anti-polymerization and anti-depolymerization of microtubules can enhance the body's immune response. The next logical step would be a better understanding of the molecular mechanism about how different tubulin-targeting agents can enhance different immunotherapeutic agents in various tumor environments. Undoubtedly, this knowledge will eventually bring about highly effective cancer therapies in individual patients [22].

Conclusion

To summarize shortly it is necessary to say that quite probably the role of tubulin as treatment target will increase constantly. It is related to quite scanty knowledge we have at this time. Of course, a great work has done to know about tubulin, but I think it almost nothing, because there are a large number of comprehensive interactions between numerous cellular structures and cascades. So it is a great incentive to move on.

References

1. Mariappan A, Soni K, Schorpp K, et al. (2019) Inhibition of CPAP–tubulin interaction prevents proliferation of centrosome-amplified cancer cells. *The EMBO Journal* 38(2).
2. Maeda H, Khatami M (2018) Analyses of repeated failures in cancer therapy for solid tumors: poor tumor-selective drug delivery, low therapeutic efficacy and unsustainable costs. *Clinical and Translational Medicine* 7(1): 11.
3. Loeser H, Schallenberg S, von Winterfeld M et al. (2017) High protein and mRNA expression levels of TUBB3 (class III β -tubulin) are associated with aggressive tumor features in esophageal adenocarcinomas. *Oncotarget* 8(70):115179-115189.
4. Desai A, Mitchison TJ (1997) Microtubule polymerization dynamics. *Annual Review of Cell and Developmental Biology* 13(1): 83-117.
5. Mitchison T, Kirschner M (1984) Dynamic instability of microtubule growth. *Nature* 312(5991): 237-242.
6. Parker AL, Kavallaris M, McCarroll JA (2014) Microtubules and their role in cellular stress in cancer. *Frontiers in Oncology* 4: 153.
7. Puurand M, Tepp K, Timohhina N, et al. (2019) Tubulin BII and BIII isoforms as the regulators of VDAC channel permeability in health and disease. *Cells* 8(3): 239.
8. Tepp K, Mado K, Varikmaa M, et al. (2014) The role of tubulin in the mitochondrial metabolism and arrangement in muscle cells. *Journal of Bioenergetics and Biomembranes* 46(5): 421-434.
9. Kavallaris M (2010) Microtubules and resistance to tubulin-binding agents. *Nature Reviews Cancer* 10(3): 194-204.
10. Jordan MA, Wilson L (2004) Microtubules as a target for anticancer drugs. *Nature Reviews Cancer* 4(4): 253-265.
11. Ludueña RF (1993) Are tubulin isotypes functionally significant. *Molecular Biology of the Cell* 4(5): 445-457.
12. Janke C (2014) The tubulin code: Molecular components, readout mechanisms, and functions. *Journal of Cell Biology* 206(4): 461-472.
13. McCarroll JA, Gan PP, Liu M, et al. (2010) β III-Tubulin is a multifunctional protein involved in drug sensitivity and tumorigenesis in non-small cell lung cancer. *Cancer Research* 70(12): 4995-5003.
14. Daire V, Giustiniani J, Leroy-Gori I, et al. (2009) Kinesin-1 regulates microtubule dynamics via a c-Jun N-terminal kinase-dependent mechanism. *Journal of Biological Chemistry* 284(46): 31992-32001.
15. Miller LM, Menthen A, Chatterjee C, et al. (2008) Increased levels of a unique post-translationally modified β IVb-tubulin isotype in liver cancer. *Biochemistry* 47(28): 7572-7582.

16. Rouzier R, Rajan R, Wagner P, et al. (2005) Microtubule-associated protein tau: a marker of paclitaxel sensitivity in breast cancer. *Proceedings of the National Academy of Sciences* 102(23): 8315-8320.
17. Ruksha K, Mezheyski A, Nerovnya A, et al. (2019) Over-Expression of β II-Tubulin and Especially Its Localization in Cell Nuclei Correlates with Poorer Outcomes in Colorectal Cancer. *Cells* 8(1): 25.
18. Walss C, Kreisberg JI, Ludueña RF (1999) Presence of the β II isotype of tubulin in the nuclei of cultured mesangial cells from rat kidney. *Cell Motility and the Cytoskeleton* 42(4): 274-284.
19. Lee KM, Cao D, Itami A, et al. (2007) Class III β -tubulin, a marker of resistance to paclitaxel, is overexpressed in pancreatic ductal adenocarcinoma and intraepithelial neoplasia. *Histopathology* 51(4): 539-546.
20. Kashyap VK, Wang Q, Setua S, et al. (2019) Therapeutic efficacy of a novel β III/ β IV-tubulin inhibitor (VERU-111) in pancreatic cancer. *Journal of Experimental & Clinical Cancer Research* 38(1): 29.
21. Zhang Y, Goto M, Oda A, et al. (2019) Antiproliferative aspidosperma-Type monoterpenoid indole alkaloids from *Bousigonia mekongensis* inhibit tubulin polymerization. *Molecules* 24(7): 1256.
22. Fong A, Durkin A, Lee H (2019) The potential of combining tubulin-targeting anticancer therapeutics and immune therapy. *International Journal of Molecular Sciences* 20(3): 586.