MPT0B098, A NOVEL MICROTUBULE-DESTABILIZING AGENT, DISPLAYS ANTI-ANGIOGENIC POTENTIAL VIA AKT/p70S6K/HIF-1α/VEGF SIGNALING IN LUNG CANCERS

Yun-Ching Cheng[1], Wen-Yang Lai[2], Chi-Yen Chang[2], Wen-Yu Pan[2], Jing-Ping Liou[3], Ching-Chuan Kuo[2], Jang-Yang Chang[2]

[1]National Institute of Cancer Research, Nation Health Research Institutes, Taiwan, [2]Division of cancer research, Nation Health Research Institutes, Taiwan, [3]College of Pharmacy, Taipei Medical University

Background

Tubulin is one of the most validated and efficacious targets for cancer therapy. Indeed, microtubule-binding drugs are widely used in cancer chemotherapy and also have clinically relevant anti-angiogenic and vascular-disrupting properties to treat solid tumors. Hypoxia occurring in solid tumors is a key microenvironmental factor for resistance to treatment, tumor progression as well as angiogenesis development through up-regulation of hypoxia-Inducible Factor-1α(HIF-1α) and vascular endothelial growth factor (VEGF). VEGF up-regulation is associated with a poor response to treatment. Thus, it may be possible to take advantage of treatment-induced hypoxia by anticancer drugs that are activated under hypoxia conditions. ABT-751 is a sulfonamide derivative, have been demonstrated as a new class of microtubule inhibitor with anti-angiogenic property in clinical study. Therefore, we continued efforts to design and synthesize a series of sulfonamide derivatives with structurally novel and to evaluate their biological activities for further drug development. Among hundreds of synthetic compounds with various substituents, MPT0B098, a 7-aryl-indoline-1-benzenesulfonamide compound, was identified as a potential lead based on extremely potent cytotoxicity with good pharmacologic properties.

Purpose

The purpose of this study could be divided into the following parts: (1) To examine whether MPT0B098 exerts tubulin-targeted property in cancer cells; (2) To investigate whether MPT0B098 exhibits anti-angiogenic activity in endothelial cells; (3) To elucidate the mechanism of action of anti-angiogenic function of MPT0B098 under hypoxia condition in lung cancer cells; (4) To evaluate the in vivo anti-cancer and anti-vascular efficacy of MPT0B098 in human xenograft mouse model.

Methods

The cytotoxicity of cells to MPT0B098 was determined by methylene blue assay. Microtubule assembly assay and competition binding assay were used to examine whether MPT0B098 inhibit in vitro tubulin polymerization due to bind to colchicine-binding domain on tubulin. Microtubule dynamics in intact cells after MPT0B098 treatment were examined by Western blotting and immunofluorescence staining. Cell cycle distribution was checked by flow cytometry. The expression level of cell cycle checkpoint and apoptotic regulators was examined by Western blotting. Caspase activity was measured by the cleavage of the fluorometric substrate. Anti-angiogenic property of MPT0B098 was examined with several in vitro analysis methods using human umbilical vascular endothelial cells (HUVECs) as the source of endothelial cells. Inhibition of HUVECs migration was determined with an in vitro wound healing assay. The anti-angiogenesis potential was further evaluated with an in vitro tube formation assay. VEGF secretion in the culture medium of HUVECs was determined by using ELISA analysis. In vivo anticancer

efficacy was performed by a mouse model bearing human xenograft. The microvessel density was examined by immuno-histochemical analysis.

Results

Cytotoxic activity of MPT0B098 in a variety of human tumor cell lines has been ascertained, with IC50 values in nanomolar ranges. Tubulin-related studies clearly showed that MPT0B098 prevents microtubule assembly through binding to the colchicine-binding site of tubulin. Cell cycle analysis and Western blotting revealed that MPT0B098 caused G2/M phase arrest and apoptosis in human lung cancer cells through the caspase-dependent apoptotic pathway. In addition, MPT0B098 is effectively to suppress the tube formation and migration of HUVECs induced by VEGF. Suppression of VEGF secretion in the culture medium of HUVECs was observed in a treatment of MPT0B098. Reduction of HIF-1α and VEGF expression by MPT0B098 would be associated with AKT/p70S6K1 pathway in non-small cell lung cancer cells under hypoxia condition. In order to investigate the anti-cancer and anti-angiogenic activity of MPT0B098 in vivo, a model of nude mice bearing xenograft of human non-small cell lung cancer H460 was performed. Notably, the results showed that MPT0B098 significantly suppressed tumor growth and microvessel density of tumor in the preclinical xenograft mouse model.

Conclusions

Taken together, our data provide compelling evidence that the novel sulfonamide-based compound, MPT0B098, acts as a microtubule-destabilizer by targeted to the colchicine-binding site of tubulin, and exerts anti-angiogenic potential via AKT/p70S6K/HIF-1α/VEGF signaling during hypoxia condition. Furthermore, MPT0B098 exhibits strong antitumor activity in vivo, at least in part, by affecting microvessel formation. These findings indicate MPT0B098 is a promising novel anticancer drug candidate with potential for treatment of human lung malignancies.