論文の内容の要旨

論文題目　Efficacy of the Novel Tubulin Polymerization Inhibitor PTC-028 for Myelodysplastic Syndrome
(骨髄異形成症候群における微小管阻害剤 PTC-028 の効果)

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Background: Myelodysplastic Syndrome (MDS) is a clonal bone marrow disorder characterized by ineffective and clonal hematopoiesis accompanied by morphological dysplasia and variable cytopenia. There are few treatment options for MDS, and allogenic hematopoietic stem cell transplantation is the only curative option. Tubulin belongs to protein superfamily of globular proteins. Monomer tubulin can polymerize into microtubules, which play an important role in the attachment and segregation of chromosomes in various phases of cell division. Therefore, the targeting of microtubules represents a therapeutic strategy against both solid and hematological cancers. The first approved microtubule-targeted agent (MTA) by the FDA was vincristine, which has been clinically used to treat multiple types of cancers, particularly hematological malignancies. Over the past few decades, additional MTAs have been developed and received FDA approval, mostly for applications to cancer therapies. These agents have been classified by their binding sites on tubulin, which influences their roles in the inhibition or stabilization of polymerized microtubules. We herein investigated the efficacy of PTC-028, a novel microtubule polymerization inhibitor for myelodysplastic syndrome (MDS).

Method: Anti-MDS efficacy of PTC-028 was studied using human MDS cell lines and primary MDS cells in vitro. The efficacy of PTC-028 was also assessed in a xenograft mouse model of MDS using a MDS cell line. PTC-028 was synthesized at PTC therapeutics Inc. Mechanistic studies were conducted via flow cytometry and RNA sequencing.

Result: A previous study reported that PTC596 suppressed cell proliferation and induced apoptosis in AML cell lines.[5] Since MDS is regarded as a pre-leukemic stage, we examined the effects of PTC-028, another novel microtubule polymerization inhibitor, on MDS cells. PTC-028 induced the dose-dependent inhibition of cell proliferation on MDS

cell lines. Caspase 3/7 activities were significantly induced in MDS-L and SKM-1 cells in the presence of PTC-028, suggesting the induction of apoptotic cell death by PTC-028. We then isolated $CD34^+$ cells from primary MDS BM samples and investigated the efficacy of PTC-028 on primary MDS cells. The efficacy of PTC-028 in CD34+ MDS cells was also confirmed by cell proliferation assays.

To enhance the therapeutic benefits of PTC-028 on MDS cells, we investigated synergism between PTC-028 and DNA hypomethylating agents. We treated MDS-L and SKM-1 cells with increasing concentrations of PTC-028 in combination with DNA hypomethylating agents, first-line therapeutic agents in the treatment of MDS. After 3 days of culture, cell growth was analyzed by MTS and Annexin V assays. PTC-028 synergized with hypomethylating agents, such as decitabine and azacitidine, to inhibit the growth and induce apoptosis of MDS cells.

We assessed the efficacy of PTC-028 in a xenograft mouse model of MDS using an MDS cell line, MDS-L and AkaBLI bioluminescence imaging system system, which is composed of AkaLumine-HCl and Akaluc. Recipient mice were treated with PTC-028 for 7 weeks. PTC-028 significantly inhibited the growth of MDS-L cells and prolonged the overall survival of recipient mice. Furthermore, a significant synergistic effect was observed between PTC-028 and decitabine. Mice that received combination therapy showed moderate weight loss 11 days after the initiation of the treatment, but subsequently recovered.

Mechanistically, a treatment with PTC-028 induced G2/M arrest followed by apoptotic cell death. We then investigated the effects of PTC-028 on the levels of soluble (unpolymerized) versus polymerized tubulin in MDS-L cells. Cells were treated with PTC-028 (3 and 5 μM) and paclitaxel (1 μM) for 4 hours, and cell lysates were then separated into soluble and polymerized fractions by centrifugation. The visualization of tubulin fractions by Western blotting demonstrated that the PTC-028 treatment for 4 hours resulted in the near-complete loss of polymerized microtubules. In contrast, polymerized microtubules increased in cells treated with paclitaxel, which stabilizes microtubules against depolymerization. These results indicate that PTC-028 also acts as a microtubule polymerization inhibitor.

Conclusion: Our data reveal a possible chemotherapeutic strategy for MDS by disruption of microtubule dynamics as a single agent and in combination with hypomethylating agents. The present study provides a preclinical framework for the clinical evaluation of this promising therapeutic approach to improve outcomes in MDS patients.