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学位記番号	博甲　第　　８８６０　　号
学位授与年月	平成　３０年　１０月　３１日
学位授与の要件	学位規則　第４条第１項該当（昭和２８年４月１日文部省令第９号）
審査組織	グローバル教育院
学位論文題目	Cell-based Screening of Natural Chemicals in Search of Compounds to Treat Stress, Cancer, and Old Age-related Pathologies（ストレス、癌及び老化関連病理の治療のための化合物探索における天然化学物質の細胞ベースのスクリーニング）

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Abstract of thesis

The current scenario of rapidly increasing aging society, environmental stresses and increasing incidence of a variety of pathologies including cancer and brain functions are raising serious concerns in health management and sustaining QOL in normal and diseases states. While drug development requires huge amount of time, efforts and costs, a variety of plants and natural compounds have been used for anti-stress and disease preventive potentials in worldwide traditional home medicine systems. They have recently attracted attention in research laboratories to dissect their mode of action to promote safe and economic drug development. Cancer being highly complex, extremely difficult to treat and fatal, has attracted a lot of attention. In spite of the advances made in recent years, it faces worst prognosis and hence warrant continued research to develop new natural compounds with preventive and therapeutic potentials. Uncontrolled proliferation is one of the most consistent hall-mark of cancer cells. Cell culture provides excellent model system for study of cancer cells in normal, abnormal, stressed and drug-treated environments. It offers an easy detection of cancer promoting and inhibiting drugs. A variety of anticancer drugs have been identified by cell culture studies and are currently used in chemotherapy. The latter is toxic and complicated by undesirable secondary effects. Metastasis, cancer cell’s ability to move from the primary site and invade distant tissues in the body, accompanied by emergence of drug resistant variants, is another major hurdle in cancer treatment. Accordingly, newer economic and

patient friendly anti-metastasis molecules are deemed useful in intervention of cancer cell aggressiveness and drug resistant properties.

The author has conducted research on the effect of Cucurbitacin B (a bioactive compound derived from *Helicteres angustifolia,* also known as Chinese Ginseng, roots) and Withanone (an active Withanolide derived from *Withania somnifera,* also known as Indian Ginseng, leaves) on cell proliferation of normal, cancer and stressed cells. The author used human normal fibroblasts and a variety of cancer cells. By extensive titration of safe and cytotoxic doses, the author first found that whereas aqueous extract of *Helicteres angustifolia* roots (AQHAR) was selectively toxic to cancer cells, the purified active compound Cucurbitacin B was also cytotoxic to normal cells. On the other hand, Withanone, a bioactive isolated from *Withania somnifera* leaves, was selectively toxic to cancer cells. However, it showed poor efficacy. The author combined Cucurbitacin B (Cuc) and Witahnone (Wi-N) and developed a combination of Cucurbitacin B and Withanone (1:500 molar ratio; called CucWi-N) that showed selective toxicity to cancer cells; the normal cells remained unaffected. By a variety of molecular assays on human non-small-cell lung cancer cells, he found that CucWi-N possesses remarkable anticancer potential and was relatively safe for normal cells. The author undertook extensive analysis and demonstrated that CucWi-N induced cellular senescence in cancer cells. It was mediated by activation of p53 and inactivation of laminin signaling. Low doses of CucWi-N that were not cytotoxic to cancer cells were found to inhibit migration of cancer cells. The author investigated the mechanism of inhibition of cell migration by undertaking bioinformatics and experimental tools and found that Cucurbitacin B and Withanone strongly target hnRNP-K, a DNA and RNA binding protein essential for cell migration. It regulates cell migration and plays key role in cancer cell metastasis. *In vivo* tumor growth assays in nude mice using subcutaneous xenografts and tail vein metastasis models revealed tumor suppressor and anti-metastasis activity of CucWi-N. Furthermore, the author found that whereas the combination (CucWi-N) showed cytotoxicity and inhibition of migration in cancer cells, it protected normal cells against stresses. It was also seen to induce differentiation in glioblastoma cells. Taken together, a novel combination of Cucurbitacin B and Withanone was generated that significantly and dose-dependently caused cytotoxicity to cancer cells and showed side-benefits to normal cells at low doses. Molecular analysis revealed selective cytotoxicity to cancer cells, their sensitization to environmental stressors, inhibition of cancer cell malignant properties including migration, stemness. Based on the data, the candidate proposed CucWi-N as a potential anticancer drug that warrants further mechanistic and clinical studies.

Short-term viability assays of cultured cells in 96 well plates are routinely used to determine the cytotoxicity or safety of drugs. These are often based on formation of a chromogen, generated selectively in viable cells. The quantitative measures of the chromogen in a spectrophotometer at a specific wavelength are the conventional read-outs. The innate problems of such short term cell viability assays include (i) effect of drugs is determined by cell density and hence may show inconsistent results, (ii) some drugs have slow/gradual effect and hence may escape such assays, (iii) cell morphology that reveal significant hints to molecular signaling underlining the effect of drugs cannot be effectively captured, (iv) long-term effect on viability and clonogenic potential of cells that may be more relevant to chemotherapy cannot be determined and (v) herbal extracts and the purified active components often possess intrinsic color, which interferes with

spectrophotometer estimation and may yield inconsistent and even false results. In light of the ease and importance of cell culture-based assessment of drug safety and cytotoxicity, the author attempted to combine the conventional cell-based assays in a way that allows multiple readouts (quantitative and qualitative) from the single experiment, avoids the drawbacks of color interference. The author has established a Quantitative and Qualitative Cell Viability (QCV) assay in 12 well cell culture plates. The author provided experimental evidence that QCV could overcome cell density problem, interference of color of drugs and allowed long term observations on cell morphology and clonogenicity. The assay is easy to use and provides reliable read-outs.

Abstract of assessment result

【Review】

The author developed a new combination of two natural compounds (Cucurbitacin B and Withanone) derived from Chinese ginseng and Indian ginseng, respectively. The author demonstrated that whereas Cucurbitacin B is toxic to normal cells, the combination of Cucurbitacin B and Withanone (1:500 molar ratio; called CucWi-N) was selectively toxic to cancer cells. The author investigated the molecular mechanism of anticancer properties of CucWi-N and found that it caused senescence in cancer cells that was mediated by activation of p53 and inactivation of Lamin signaling. Furthermore, inactivation of mortalin and hnRNP-K proteins contributed to decreased cell migration and metastasis of cancer cells. The author also developed a new protocol for accessing the cytotoxicity/viability of compounds possessing color. The author demonstrated that his protocol yields reliable results that are not affected by either cell density, growth characteristics of cells or even the innate color of test compounds. The study passes the criteria of originality, well planning and accomplishment. The objective of research is clear and well understood. Experimental models used are well suited. Interpretation of the results is clear and reasonable. Data analyses have been done using appropriate tools. The results and discussion sections are well written and interpreted. The study contributes significantly in the field of innovation and disease mechanisms with special reference to understanding of cancer and its interventions.

【Result】

The final examination committee conducted a meeting as a final examination on 4 July, 2018. The author provided an overview of dissertation, addressed questions and comments raised during Q&A session. All of the committee members reached a final decision that the applicant has passed the final examination.

【Conclusion】

Therefore, the final examination committee approved that the applicant is qualified to be awarded Doctor of Disease Mechanism.