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学位の種類	博　士（環境学）
学位記番号	博　甲　第　9872　号
学位授与年月日	令和　3　年　3　月　25　日
学位授与の要件	学位規則第４条第１項該当
審査研究科	生命環境科学研究科
学位論文題目	Study on the Molecular Mechanism and Enhancement of Anticancer Activity of Ashwagandha and Propolis Derived Natural Compounds by Nanoparticles （アシュワガンダおよびプロポリス由来のナノ粒子化した天然化合物の抗癌活性の強化及びその分子メカニズム）

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論　文　の　要　旨
Abstract of thesis

Many natural medicines and their derivative compounds have been identified as potential chemopreventive agents which can enhance immune function, speed up recovery, reduce toxicity associated with radiochemotherapy, improve quality of life and prolong survival, and hence are popular as complementary treatments. Previous studies show that alcoholic extract of Ashwagandha leaves (i-Ex) and caffeic acid phenethyl ester (CAPE) possess potent anticancer activity both in *in vitro* and *in vivo*. However, the clinical applications of these natural products are still limited due to the lack of targeting capability, as these molecules cannot distinguish normal cells from tumor cells leading to severe toxicity to normal cells. Their poor aqueous solubility is another major challenge that hampers their clinical applications. Recently the rapid development of nanotechnology has provided the new possibilities of efficient delivery and specific targeting of chemotherapy drugs to tumor cells. This research for the first time attempted to increase the anticancer potency of i-Ex and CAPE by recruiting nanoparticles employing folic acid and anti-mortalin antibody (MotAb), respectively.

This dissertation is divided into 4 chapters. In Chapter 1, the author presented literature review on the bioactivities of Ashwagandha, propolis, and their derivatives. In this chapter, the author addressed the major challenges of the pharmaceutical formulation of hydrophobic drugs derived from natural source. Specifically, the author addressed the great potential of nanoparticle-based targeted drug delivery in achieving higher efficacy of natural medicines, especially in cancer therapy. Research objectives, innovative points and the

framework of this research were arrived at the end of this chapter. In Chapter 2, the author investigated the enhanced anticancer activity of Ashwagandha leaf extract by targeting folate receptor. Results show that folate receptor-targeting i-Ex nanocomplexes (FRi-ExNC) suspended well in water. The encapsulation efficiency of i-Ex was increased up to 87%, when i-Ex and 1, 2-distearoyl-sn-glycero-3-phosphoethanolamine-poly(ethylene glycol) (DSPE-PEG) were used at a 1:3 ratio. i-Ex uptake efficiency was increased 2.5 folds (from 0.76 of i-ExNC to 1.89 of FRi-ExNC) in folate receptor (FR)-positive HeLa cells but remained unchanged in FR-negative MCF7 cells. Comparative analysis of FR-positive and -negative cells revealed that FRi-ExNC caused a stronger decrease in Cyclin D1/Cdk4 and anti-apoptotic protein Bcl-2, as well as a higher increase in the growth arrest regulating protein $p21^{WAF1}$ and pro-apoptotic protein PARP-1, in FR-enriched cancer cells. The subcutaneous xenograft nude mouse model showed that the tumor inhibition rate in response to FRi-ExNC treatment reached 63.1% in the HeLa xenografts, which was much higher than those treated with i-Ex (18.4%) and i-ExNC (31.2%). In Chapter 3, the author researched the enhanced anticancer activity of CAPE by targeting mortalin. Results showed that mortalin-targeting CAPE nanoparticles (CAPE-MotAb) were water-soluble and internalized by the cells. The encapsulation efficiency of CAPE reached the highest value of 84.88% ± 8.66% at 1:20 ratio of CAPE to 3-(N-succinimidyloxyglutaryl) aminopropyl, polyethyleneglycol-carbamyl distearoylphosphatidyl ethanolamine (DSPE-PEG-NHS). The loading efficiency of CAPE reached the highest value of 19.65% ± 0.96% when CAPE and DSPE-PEG-NHS were used in a 1:1 ratio. CAPE uptake efficiency was increased 2.2 folds (from 6.9 of CAPE-PEG to 14.9 of CAPE-MotAb) in A549 cells. CAPE-MotAb caused a stronger dose-dependent growth arrest/apoptosis of cancer cells through the downregulation of Cyclin D1/Cdk4, phospho-Rb, PARP-1 and anti-apoptotic protein Bcl2. Concomitantly, a significant increase in the expression of p53, $p21^{WAF1}$ and caspase cleavage was detected only in CAPE-MotAb treated cells. CAPE-MotAb caused a remarkably enhanced downregulation of proteins critically involved in cell migration. *In vivo* tumor growth assays for subcutaneous xenografts in nude mice also revealed a significantly enhanced suppression of tumor growth in the treated group. Finally, in Chapter 4, the author summarized the major conclusions of this study, and proposed the future research directions.

審　査　の　要　旨
Abstract of assessment result

This research attempted to enhance the anticancer activity of i-Ex and honeybee propolis ingredient, CAPE by generating two different types of nanoparticles employing folic acid (FRi-ExNC) and anti-mortalin antibody (CAPE-MotAb). These newly generated FRi-ExNC nanoparticles achieved encapsulation efficiency of i-Ex up to 87%, 2.5 folds increase in uptake efficiency of i-Ex. With the aid of folate ligands, FRi-ExNC induced stronger cytotoxicity to folate receptor (FR)-α-enriched cancer cells *in vitro* and stronger tumor suppression of FR-α-positive xenografts. In addition, the newly developed CAPE-MotAb nanoparticles were also found to possess enhanced targeted delivery and selective cytotoxicity to cancer cells both *in vitro* and *in vivo*. These novel FRi-ExNC and CAPE-MotAb nanoparticles could be proposed as suitable nanomedicines and platforms to facilitate the use of i-Ex and CAPE for cancer treatment and hence warrant clinical trials. This study provides scientific reference for the enhancement of anticancer activity of natural compounds like i-Ex and CAPE by recruiting nano vehicles, which also opens more opportunities for designing targeted drug delivery systems to improve the poor bioavailability of these molecules derived from natural resources and reduce their effect on normal cells.

The final examination committee conducted a meeting as a final examination on 15 January, 2021. The applicant provided an overview of the dissertation, addressed questions and comments raised during Q & A session. All the committee members reached a final decision that the applicant has passed the final examination.

Therefore, the final examination committee approved that the applicant is qualified to be awarded the degree of Doctor of Philosophy in Environmental Studies.