

公益財団法人富山第一銀行奨学財団

理事長 金岡 純二 殿

助成研究成果概要報告書

教育機関名 : 富山大学	助成金額 :	1,000 千円
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研究題目 : 抗膵臓がん活性を有する有望な植物資源としての富山県産檜に関する天然物化学研究		

研究概要

富山県が、我が国の伝統薬産業及び伝統医薬研究をリードしていることは周知の事実である。この事実を踏まえ、本課題は、申請者らが確立してきた栄養飢餓戦略に基づき富山県の有する天然物資源から新規抗腫瘍薬の開発に取り組むものである。特に、今日、膵臓がんに焦点をあてた申請者らの研究では、富山県に自生する檜からの抽出物に抗膵臓がん活性を認め、それが新規の治療薬ソースになる可能性を見出している。

成果要約

The chloroform extract of the Japanese cypress *Chamaecyparis obtuse* also known as “Hinoki” collected from Toyama Prefecture was found to kill PANC-1 human pancreatic cancer cells preferentially in the nutrient-deprived medium without causing toxicity in the normal nutrient rich condition. Phytochemical investigation on this extract led to the isolation of two new germacrane-type sesquiterpenes (**1**, **2**), together with the six sesquiterpenes (**3-8**) and a lignan (**9**).

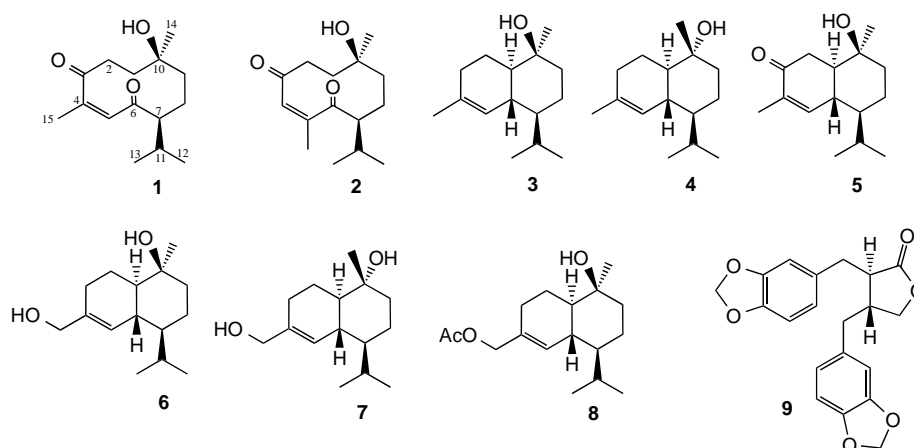


Figure 1. Structure of compounds isolated from “hinoki”

The isolated compounds were tested for their preferential cytotoxicity against six different human pancreatic cancer cell lines [PANC-1, MIA PaCa2, KLM-1, NOR-P1, CAPAN-1 and PSN-1] by utilizing an antiausterity strategy. Among these, α -cadinol (**3**) was identified as the most active

constituent having PC_{50} values of 22.8 μM (PANC-1), 19.3 μM (MIA PaCa-2), 31.0 μM (CAPAN-1), 31.6 μM (PSN-1), and 45.1 μM (KLM-1), respectively.

The most active compound, α -cadinol was further evaluated for its effect in real-time against PANC-1 cells using electronic impedance-based cell analyzer. PANC-1 cells cultured in only NDM (control) showed increased cell index value even after 24 h of starvation. However, treatment with α -cadinol was found to inhibit the cellular index in a concentration dependent manner (fig. 2).

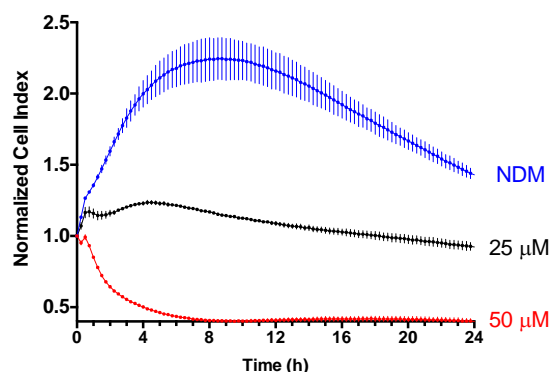


Figure 2. Real-time cell monitoring of PANC-1 cells treated with α -cadinol in NDM. The normalized cellular index of PANC-1 cells at 24 hours of treatment (T_{24}) with 25 μM of α -cadinol was found to be the same as initial level (T_0) suggesting the complete inhibition of proliferation. α -cadinol at the concentration of 50 μM , however lead the quick decrease in the PANC-1 cell index value within 4 h, and total cell death within 8 h.

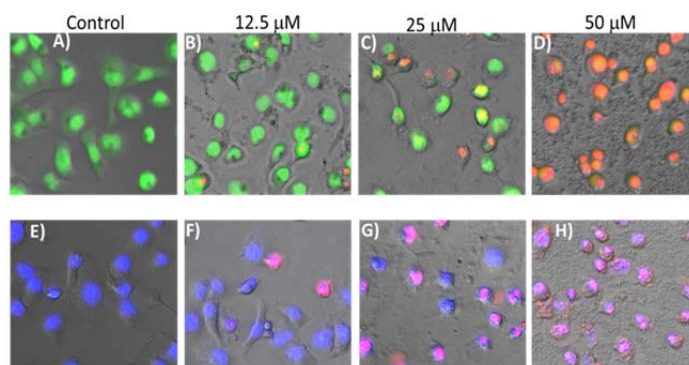


Figure 3. α -Cadinol induced morphological alteration against on PANC-1 human pancreatic cancer cells. I) Fluorescent EB/AO staining [Red: Ethidium Bromide (EB) dead cells' indicator Green: Acridine Orange (AO) live cells' indicator] and phase contract overlay image. II) Fluorescent Hoechst 33342/PI staining [Blue: Hoechst is cell permeable dye, stains the condensed chromatin in apoptotic cell more brightly than in normal cells, Pink: Propidium iodide (PI) only permeant to dead cells] and phase contract overlay image.

The α -cadinol was further studied for its effect on cell morphology and apoptosis using ethidium bromide (EB)/acridine orange (AO) and Hoechst 33342/propidium iodide double staining assays. As shown in figure 3, control cells showed intact cell morphology with bright green AO

fluorescence in EB/AO and flattened rounded blue nuclei in Hoechst 33342/PI staining. Cells treated with α -cadinol showed the concentration dependent increase in dead cells. However, nuclear fragmentation was not observed in Hoechst 33342/PI staining, suggesting α -cadinol induced the necrotic cell death against PANC-1 cells in NDM.

Akt is a pro-survival factor that is activated in a majority of tumors and regulates cellular functions such as cell cycle progression, cell migration, invasion, and angiogenesis. Increased Akt phosphorylation is one of the austerity markers that enable tumor cells to survive and proliferate in hostile hypovascular tumor environment. Therefore, the effect of α -cadinol on Akt activation under nutrient-deprived conditions was investigated by Western blot analysis. As shown in Figure 6, PANC-1 cells in showed high level of p-Akt. However, treatment with α -cadinol dramatically inhibited p-Akt expression within 3 h (Fig. 4). α -cadinol was also found to inhibit the major downstream effector of Akt activation, mTOR (mammalian target of rapamycin).

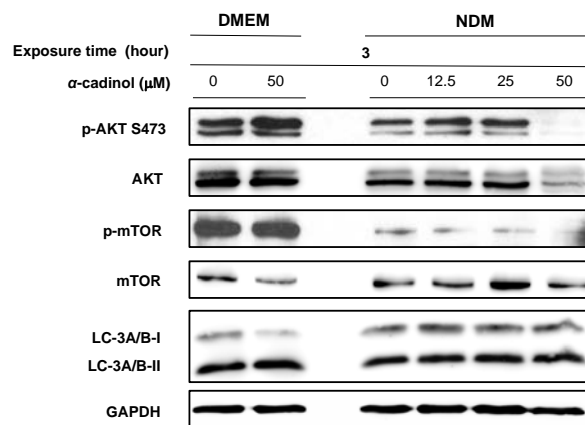


Figure 4. Effect of α -cadinol on Akt, mTOR, LC3A/B-I and LC3A/B-II by Western blot.

Autophagy is a cellular recycling process to maintain the energy metabolism, and is often activated in response to various stresses, including nutrient deprivation in the tumor microenvironment. Therefore, we examined the expression of autophagic markers, microtubule associated protein light chain LC3-I (16 kDa) and LC3-II (14 kDa). As shown in figure 6, no apparent differences were observed in the LC3-I and LC3-II expression in the controls. However, treatment with α -cadinol led to significant enhancement in the expression of both LC3-I and LC3-II in a concentration dependent manner. Therefore, hyperactivation of autophagy, together the inhibition of p-Akt (S⁴⁷³) and p-mTOR expression by α -cadinol could have contributed to the preferential cytotoxicity of PANC-1 cells during nutrition deprived condition. Human pancreatic cancer cells are inherently resistant to conventional anticancer drugs in clinical use such as gemcitabine and 5-fluorouracil. Therefore, α -cadinol isolated from the “Hinoki” collected in Toyama should be an attractive candidates antiausterity drug development.

<p>研究成果 発表状況</p>	<p>【雑誌論文、学会発表、図書、新聞掲載、作成 Web ページ、特許権等の出願・取得状況】</p> <p>学会発表 Dya Fita Dibwe, Sijia Sun, Jun-ya Ueda, Matsumoto K., <u>Suresh Awale</u> : Cadinane-type sesquiterpenes from <i>Chamaecyparis obtuse</i> and their antiausterity activity 日本薬学会第 137 年会, 2017, 3/26-29, 仙台.</p> <p><u>Suresh Awale</u> : Recent advances in antiausterity strategy guided anticancer drug discoveries The 5th International Symposium on Bioactivities and Disease Prevention of Phytochemicals and Natural Products for Health, National Chiayi University Chiayi, 2016, 11/25-26, 台湾.</p> <p>雑誌論文 Dya Fita Dibwe, Jun-ya Ueda, Chandrasekar Balachandran, Sijia Sun, Matsumoto K., <u>Suresh Awale</u> : Cadinane-type sesquiterpenes from <i>Chamaecyparis obtuse</i> and their antiausterity activity, (in preparation, to be submitted in April, 2017)</p>																													
<p>経費の 執行状況</p>	<table border="1"> <thead> <tr> <th data-bbox="322 1205 810 1258">区 分</th> <th data-bbox="810 1205 1114 1258">執行額 (円)</th> <th data-bbox="1114 1205 1437 1258">備 考</th> </tr> </thead> <tbody> <tr> <td data-bbox="322 1258 810 1361">【物品費】</td> <td data-bbox="810 1258 1114 1361"></td> <td data-bbox="1114 1258 1437 1361"></td> </tr> <tr> <td data-bbox="322 1361 810 1406"> 有機溶媒類</td> <td data-bbox="810 1361 1114 1406">163,539 円</td> <td data-bbox="1114 1361 1437 1406"></td> </tr> <tr> <td data-bbox="322 1406 810 1451"> アッセイ用試薬類</td> <td data-bbox="810 1406 1114 1451">490,628 円</td> <td data-bbox="1114 1406 1437 1451"></td> </tr> <tr> <td data-bbox="322 1451 810 1554"> 機器使用コスト (NMR、MS、CD など)</td> <td data-bbox="810 1451 1114 1554">263,713 円</td> <td data-bbox="1114 1451 1437 1554"></td> </tr> <tr> <td data-bbox="322 1554 810 1599">【旅費】</td> <td data-bbox="810 1554 1114 1599">82,120 円</td> <td data-bbox="1114 1554 1437 1599"></td> </tr> <tr> <td data-bbox="322 1599 810 1644">【謝金】</td> <td data-bbox="810 1599 1114 1644">0 円</td> <td data-bbox="1114 1599 1437 1644"></td> </tr> <tr> <td data-bbox="322 1644 810 1688">【その他】</td> <td data-bbox="810 1644 1114 1688">0 円</td> <td data-bbox="1114 1644 1437 1688"></td> </tr> <tr> <td data-bbox="322 1688 810 1917">合計</td> <td data-bbox="810 1688 1114 1917">1,000,000 円</td> <td data-bbox="1114 1688 1437 1917"></td> </tr> </tbody> </table>	区 分	執行額 (円)	備 考	【物品費】			有機溶媒類	163,539 円		アッセイ用試薬類	490,628 円		機器使用コスト (NMR、MS、CD など)	263,713 円		【旅費】	82,120 円		【謝金】	0 円		【その他】	0 円		合計	1,000,000 円			
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