樟芝，芭樂心葉對人類攝護腺癌細胞株之生長抑制與其機轉探討

Growth Inhibition and Its Mechanisms of Antrodia camphorata and Psidium gujava L. Leaf on Human Prostate Cancer Cell Lines

中文摘要

攝護腺癌中以腺癌(Adenocarcinoma)最爲常見。攝護腺癌在美國之男性泌尿生殖系統的癌症死亡原因中居第二大位，而在台灣目前位居第八位，並且也在 2004 年以年增率 10.38% 佔十大癌症年增率之冠。

樟芝 (Antrodia camphorata) (novel name: Taiwanofungus camphoratus) 是一種獨特而且具有許多醫療用途的真菌植物，只生長在台灣特有的牛樟樹上，因此屬於稀少且昂貴的保育類植物之一。長久以來，曾經被用來當做藥物中毒、腹瀉、腹痛、高血壓及肝癌等的治療處方。芭樂心葉 (Psidium guajava)則在民間沿用已久，並在有效治癒許多疾病方面有許多報告，包括殺菌、抗腹瀉、抗寄生蟲、止痛、 抗發炎、抗痙、鎮靜、滋補、黃膽和其他惡病質壞血病和潰瘍。近年來包括中草藥治療的另類療法(Complementary Alternative Medicine, CAM) 逐漸興起，我們嘗試探討樟芝及芭樂心葉是否具有抑制攝護腺癌細胞生長之作用，並進一步希望了解其作用機制。我們利用樟芝子實體(Fruiting bodies)粗萃取物分別作用於兩株雄性素依存程度不同的攝護腺癌細胞株 (LNCaP 及 PC3)，並利用老鼠肧胎纖維母細胞 (MEF) 作爲實驗之對照組。另一株非雄性素依存性攝護腺癌細胞株 DU145 則以芭樂心葉水萃取物做處理，並利用攝護腺腺體上皮細胞株 PZ-HPV-7 作爲實驗之對照組。在樟芝子實體粗萃取物最低有效劑量 150μg/mL的作用下，LNCaP 細胞顯示 G1/S 期之生長停止，並有明顯之細胞自然死亡。這種劑量依存的反應方式同時被證實是經過Akt活化 p53進而活化 p21進而抑制 CDK4/Cyclin D1後造成 G1/S 期生長停止且導致細胞自然死亡之訊息傳遞路徑所產生，同時並涉及 Cyclin D1 活性之抑制及防止 pRb 之磷酸化。相反地，在沒有 p53 表現之情況下，PC3 細胞則顯示 G2/M 期之停止，經由活化 p21 進而抑制 Cyclin B1/Cdc2 後造成 G2/M 期之停止，但只有少量之細胞自然死亡。這些現象顯示樟芝子實體粗萃取物能藉由調控不同之細胞週期訊息傳遞路徑來抑制不同特性之攝護腺癌細胞生長。

另外在芭樂心葉水萃取物 1.0 mg/mL的劑量下，芭樂心葉水萃取物在作用於 DU145 細胞達 48 及 72 小時之後分別可使細胞存活度下降。芭樂心葉水萃取物在作用於DU145 細胞達 72 小時之後其絕對細胞存活度抑制能力達到 262.5 細胞數-毫升-小時/毫克。另外，在芭樂心葉水萃取物之作用下，DU145 細胞之菌落形成能力也明顯偏低。藉由 TUNEL 分析和流式細胞儀分析可觀察到細胞週期在 G0/G1 期停止的現象。另外，芭樂心葉水萃取物對於 DU145 細胞之間質

金屬結合蛋白脢ＭＭＰ-2 及ＭＭＰ-9 之抑制及活化性 caspase-3 之正向調控呈現劑量依存的反應方式，這意味芭樂心葉水萃取物具有潛在之抗轉移能力。
總而言之，樟芝可以用來抑制雄性素依存程度不同的攝護腺癌細胞 LNＣaP 及 PC３。另一方面，芭樂心葉水萃取物的抗癌活性則歸因於其成分內特高之多酚類物質及類黃酮素，可有效抑制大腦轉移性之攝護腺癌細胞ＤＵ１４５。

英文摘要

Prostate cancer (PCa) has been cited to result from the neoplastic lesion with genetic and/or environmental factors identified as causatives. Among which the adenocarcinoma is the most common. PCa has caused the top second mortality of cancer frequently encountered by males in the United States, and overall, it is the top eighth cancer mortality in Taiwan with the top increment of 10.38% in 2004.
Antrodia camphorata (Polyporaceae) (AC), a unique mushroom that grows merely on the inner bark of a characteristic species of camphor tree called Cinnamomum kanehirai, is a very rare and valuable ecologically protected plant. Since long ago, it has been adopted for treatment of intoxication, diarrhea, abdominal pain, hypertension, and hepatoma. Psidium guajava, as a folkloric medicines, have been reported to be effective in curing many diseases involving bacteriocidal , antidiarrhea, antiparasitic, analgesic, antiinflammation, antispasmodic, antipyretic, sedative, tonic, jaundice; and other cachexia, scurvy and unhealthy ulcers.
Recently, the development of the so-called Complementary Alternative Medicine (CAM) has strongly attracted us to investigate the potential of anti-prostate cancer of AC and Psidium guajava. In this study, prostate cancer cell lines PC-3 (androgen independent) and LNCaP (androgen responsive) were used to treat with AC crude extract (ACCE). The mouse embryonic fibroblast cells (MEF) was used as the control cells. Another prostate cancer cell line DU-145 (androgen independent) was treated with the aqueous extract of Psidium guajava L. and PZ-HPV-7 cells were serve as the normal prostate control cells.
At the minimum effective dose of 150 μg/mL ACCE, treated LNCaP showed a G1/S phase arrest with significant apoptosis. The dose dependent behavior of LNCaP cells in response to ACCE was identified to process an Akt apoptosis pathway, in which Cyclin D1 activity and pRb phosphorylation were both down-regulated. In contrast, being without p53, PC3 cells showed a G2/M phase arrest mediated through the p21 proceed Cyclin B1/Cdc2 pathway, however, with limited number of cell apoptosis observed. These results implicate that ACCE is able to differentially inhibit the growth of different cells by modulating different cell cycle signaling pathways.
After treated with 1.0 mg/mL of PE, the PE showed a viability suppressing capability (VSC)AC of 262.5 cells-mL-h/mg on DU-145 cells. In addition, the colony forming

capability of DU-145 cells was apparently decreased. Cell cycle arrests at G0/G1 phase was observed by TUNEL assay and flowcytometric analysis. Furthermore, suppression of the matrix metalloproteinases MMP-2 and MMP-9, and the upregulation of active caspase-3 were also identified in a dose-dependent manner by PE. Our results implicate a potent anti-metastasis power of PE.

We conclude that the unique Formosan mushroom AC, because of being nontoxic, might be used as a good adjuvant anti-cancer therapy for PCa despite of its androgen responsive behaviors. In the other aspect, we ascribe the anticancer activity of PE to its extraordinarily high polyphenolic and flavonoid contents. Furthermore, PE might be useful for treatment of brain derived metastatic cancers such as DU-145, acting simultaneously as both a chemopreventive and a chemotherapeutic.