Discussion

An understanding of the factors regulating the expression and function of lipid biosynthesis enzymes in cancer cells is importance both from the clinical and toxicological perspectives. In earlier studies have demonstrated that cancer cells display an excessive fatty acid genesis. In contrast, nearly all non-malignant adult tissues have very low lipogenesis (Meadows AL et al., 2008, DeBerardinis RJ et al., 2007). Lipids are required for membrane synthesis as well as the composition of membrane microdomains of cancer cells. Membrane microdomains or lipid rafts including of phosphlipids, cholesterol and sphingolipids have been reported to be involved in several cellular processes such as signal transduction, intracellular trafficking, cell polarization, and cell migration (Bagnat M and Simons K, 2002, Manes S et al., 1999, Ikonen E and Simons K, 1998). Inhibition of enzymes contributing in tumor fatty acid biosynthesis results in decreasing fatty acid accumulation that leads to depressing cell proliferation, loss of cell viability and decreasing tumor size (Brusselmans K et al., 2005, Hatzivassillou G et al., 2005, Pizer ES et al., 1996). However, decreased lipid biosynthesis by inhibition of enzymes of fatty acid synthesis in non-malignant human fibroblast cells does not influence on both cell proliferation and viability (Chajes V et al., 2006, Brusselmans K et al., 2005, Pizer ES et al., 1996, Kuhajda FP et al., 1994).

The present study further examined the effects of piperine on lipid biosynthesis in HepG2 cells. Piperien at 1 mmole/L for 24 h was capable of decreasing cell proliferation approximately 50%, while piperine at 400 μmole/L for 48 h and at 100 μmole/L for 72 h or more than was capable of reducing cell proliferation more than 50%. The decreasing cell proliferation was shown by apoptosis induction experiments to further determine the cause of cell death. Our data demonstrate piperine is efficacious in inhibiting the proliferation of HepG2 cells, most likely by induction of apoptosis in a manner that is time- and concentration-dependent. Piperine-induced cytotoxicity was dependent on FASN expression. We found that FASN expression was decreased after 24 and 48 h of piperine treatment. The mechanism of action by which piperine induces inhibition of FASN expression remains poorly understood, however some possibilities have been proposed. Piperine might affect to decrease cytosolic citrate levels, the substrate provided for acetyl CoA synthesis that leads to an impairment of fatty acid synthesis. The reducing cytosolic citrate levels affects mitochondrial homeostasis and

membrane potential, presumably through the decreased availability of mitochondrial citrate for TCA cycle.

More than half a century ago, studies have shown that the majority of fatty acids in cancer cells are derived from de novo fatty acid synthesis whereas most other normal cells acquire the majority of fatty acids from the circulation. This increased lipogenesis is reflected in a significantly elevated expression and activity of lipogenic enzymes, ACL, FASN, and ACC. Enzyme ACL provides the requisite substrate acetyl-CoA for fatty acid synthesis by catalyzing the conversion of citrate to acetyl-CoA. ACL inhibition suppresses the growth and survival of tumor cells, although the mechanisms of ACL inhibition-dependent cell death are still not clear.

Treating cells with piperine inhibited FASN expression and induced apoptosis. Several studies reported that inhibition of FASN did not affect growth rate or viability of nonmalignant cells. These date indicate that FASN predominantly plays role in tumor growth and survival. Although the precise mechanisms of FASN inhibition-induced cells death in cancer cells still remain unknown, several possibilities have been proposed. Initial studies indicate that FASN inhibition accumulates the toxic intermediary metabolites, malonyl CoA, which indices apoptosis, whereas pharmacological inhibition of acetyl-CoA carboxylase (ACC) enzyme by 5-(tetradocyloxy)-2-furoic acid (TOFA) does not (Pizer ES., et al., 2000). The acute reduction of fatty acid production, palmitate, *per se* was not the major source of cell injury after FASN inhibition. The high levels of malonyl-CoA were a characteristic effect of FASN inhibitors and temporally preceded the other cellular responses, including apoptosis. TOFA rescued cells from FASN inhibition by reducing malonyl-CoA accumulation. These studies suggest that malonyl-CoA accumulation, but not end product fatty acid depletion, could be a critical factor for apoptosis induction. These results also suggested that malonyl-CoA accumulation may not by a significant problem in normal tissues, possibly because fatty acid synthesis pathway activity is normally low, even in the lipogenic organs, such as liver. Physiologically, the elevated levels of malonyl-CoA occurring during fatty acid synthesis reduce fatty acid oxidation, by inhibiting carnitine palmitoyltransferase I at the outer mitochondrial membrane, to prevent a futile cycle of simultaneous fatty acid synthesis and degradation. However, the mechanisms of high levels of malonyl-CoA lead to apoptosis is not yet known, carnitine palmitoyltransferase I, which is regulated by malonyl-CoA, has been shown to interact directly with Bcl-2 at the mitochondrial

membrane. Thus, high levels of malonyl-CoA may either induce apoptosis directly or alter mitochondrial mechanism to increase susceptibility to apoptosis from other signals.

However, studies with siRNAs reveals that ACC inhibition triggers apoptosis in some cancer cells, but not in normal cells. Inhibition of ACC expression results in a major decrease in the cellular pool of palmitic acid and apoptosis induction. However, supplementing the culture medium with palmitic acid completely rescues cell from both FASN and ACC-induced apoptosis. These finding confirm the importance of end-product fatty acid starvation to cell death. Moreover, besides the role of FASN-dependent fatty acid synthesis in fatty acid supply for membrane production and energy production through β–oxidation, FASN overexpression is further associated with palmitoylation and lipid modifications of signaling proteins. Thus, interference FASN expression could also be involved FASN inhibition-induced selective cancer cytotoxicity. Moreover, inhibition of FASN has been shown to induce endoplasmic reticulum stress in tumor cells, and a further mechanism of action may involve cooperation with endoplasmic reticulum stress inducers to enhance apoptosis. In addition, as with FASN inhibition, piperine blocked the cell cycle in G1/G0 phase. Thus, this finding re-emphasizes the essential role of piperine-induced FASN inhibition associated with cell cycle blockage.

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Output จากโครงการวิจัยที่

1. ผลที่ได้จากโครงการวิจัยนี้สามารถที่จะตีพิมพ์ได้ในวารสารระดับนานาชาติที่เป็นที่ยอมรับได้ อย่างน้อย 1 เรื่อง ซึ่งนักวิจัยกำลังอยู่ในช่วงเขียน manuscript เพื่อ submit ลง journal

- Toxicology and Applied Pharmacology year 2012 หรือ
- Journal of Ethnopharmacology year 2012

2. การนำผลงานวิจัยไปใช้ประโยชน์
   - เชิงวิชาการ ผู้วิจัยเองได้รับความรู้และประสบการณ์ในการทำวิจัยทั้งระบบ อันเป็นประโยชน์ต่อการเรียนการสอนในระดับปริญญาตรี โท และเอก และยังเป็นพื้นฐานในการทำวิจัยร่วมกับสาขาอื่นในลักษณะที่ครบวงจร ทั้งนี้ผู้วิจัยได้ต่อยอดงานวิจัยนี้ โดยนำผลงานวิจัยนี้ไปสร้างนักวิจัยรุ่นใหม่ โดยได้เขียนโครงงานวิจัยร่วมกับนิสิตป.โทที่ปรึกษา เกี่ยวกับการใช้สารสกัดจากสมุนไพรมาเป็นตัวยับยั้งการเจริญของเซลล์มะเร็ง โดยศึกษาผลของสารต่อการยับยั้งการใช้ citrate ในการสร้าง de novo fatty acid ภายในเซลล์มะเร็ง
   - เชิงสาธารณะ ซึ่งได้มีการสร้างความร่วมมือกับคณาจารย์ นักวิจัยภายในคณะ และคณาจารย์ นักวิจัยจากคณะเภสัชศาสตร์และคณะวิทยาศาสตร์ ร่วมกันเขียนโครงงานวิจัย โดยศึกษาผลของสารสกัดจาก bacteria actimycetes มาเป็นสารยับยั้งการสร้าง fatty acid ในเซลล์มะเร็ง โดยมุ่งเน้นไปที่ผลต่อการลด citrate ภายในเซลล์ และส่งโครงงานวิจัยนี้เพื่อขอทุนสนับสุนจากแหล่งทุนทั้งภายในมหาวิทยาลัย และภายนอกมหาวิทยาลัย ซึ่งกำลังอยู่ในระหว่างการพิจารณาให้ทุนจากแหล่งทุนดังกล่าว