CHAPTER I
INTRODUCTION

Lung cancer has been recognized as a major health problem worldwide, and approximately 90 % of the death found in this type of cancer is associated with metastasis (1-3). During the process of metastasis, the cells must escape from apoptosis triggered by cell detachment term "anoikis" (3). Anoikis is a fundamental process to eliminate adherence cells after they lose appropriate contact to extracellular matrix (ECM) (4). To spread, the detached cancer cells have to survive in the blood or lymphatic circulations until they establish themselves as secondary tumors. Because the process of anoikis has received considerable attention and accepted as a critical mechanism to inhibit the primary cancer cells spreading, the anti-metastasis approaches focusing on enhancement of anoikis response of the cells have been continuously demonstrated (2, 3)

Several studies reveal roles of apoptosis-regulatory protein in Bcl-2 family in many cancers. The down-regulation of anti-apoptotic proteins such as Mcl-1 (5-7), Bcl-2 (8, 9) was shown to enhance anoikis response in cancer cell lines, while the over-expression of Bcl-2 in human osteosarcoma cells prevented suspended cells from anoikis (10). In addition, the up-regulation of pro-apoptotic Bax protein was shown to increase apoptosis triggered by the loss of cell adhesion (5, 7). So far, compounds from natural sources that have potential to be developed as anti-metastasis agents, such as, curcumin (8), imperatorin (5), ecteinascidin (7), renieramycin M (11), and artonin E (6) have been shown to mediate anoikis sensitization through down-regulation of such anti-apoptotic proteins. Recently,

caveolin-1 (Cav-1), an essential protein of the caveolae (12), has been shown to associate with poor prognosis in various types of cancer (13-15). The up-regulation of Cav-1 is associated with an enhancement of cancer metastasis, invasive capacity, and progression (15, 16). Cav-1 has been shown to implicate in anoikis resistance (17-20). A study by Rungtabnapa et al. indicated a novel finding that hydrogen peroxide can prevent ubiquitination and proteosomal degradation of Cav-1 after cell detachment, and therefore induce anoikis resistance (20). The increased or sustained level of Cav-1 was shown to increase activated (Protein kinase B) Akt level in detached cells and is responsible for the survival of detached cancer cells (21). In addition, the Cav-1 protein was shown to interact and stabilized the level of Mcl-1 in lung cancer cells (22).

Cancer migration was shown to be a critical early step for achieved metastasis (23). Previous studies reported that the suppression of cancer migration could inhibit cancer dissemination (24-26). Indeed, migration of cancer cells is a multi-step process that requires the functions of focal adhesion kinase (FAK) and Rho family including Rac and Rho (27). The increase level of activated FAK (pFAK) has been found in the migrating cancer cells (28, 29), while suppression or the decrease of pFAK level could inhibit cancer cell motility (24-26). The activated FAK was shown to promote cell migration through the PI3K/Akt-dependent pathway (30) that in turn promote the activations of Rho and Rac proteins (27). Both Rho and Rac induce actin remodeling, an important process for control the cell migration (31).

In line with the previous studies, we aimed to investigate the possible anoikis sensitizing and anti-migration effects of dendrofalconerol A or DF-A (4,6-Dimethoxy-9-(4-methoxybenzyl)-8-[2-(4-methoxyphenyl)ethyl]-9H-xanthene-2,3,5-triol), a pure

compound isolated from *Dendrobium falconeri* (Orchidaceae) (32). The findings gained from the present study may benefit the development of this compound to be used as anti-metastasis agent as well as encourage the investigation of compounds to be used in such approaches.

Research Questions

1. Can Dendrofalconerol A (DF-A) sensitize anoikis and inhibit migration in lung cancer H460 cells?
2. What are the underlying intracellular mechanisms that DF-A sensitize anoikis and inhibit migration in lung cancer H460 cells?

Objectives

1. To investigate the effect of DF-A on lung cancer H460 cells anoikis and inhibitory effect of DF-A on lung cancer cell migration.
2. To study intracellular mechanism that DF-A sensitize anoikis of lung cancer H460 cells and migratory of DF-A in lung cancer cell migration.

Hypothesis

DF-A sensitizes anoikis of lung cancer H460 cells by suppressing Akt-mediated survival pathway, decreasing Bcl-2 protein, and Cav-1 protein. Also, DF-A is able to inhibit lung cancer migration through suppressing the migratory-related proteins including pFAK, Rho-GTP and Rac-GTP signaling pathway.