CHAPTER V
DISCUSSION AND CONCLUSION

Metastasis of lung cancer is recognized as an important cause of cancer-related death worldwide. To metastasis, disseminating cancer cells must have an ability to survive after detachment. The cells then transport through the circulating systems, and establish themselves at secondary sites (2). The development of secondary tumors accompanies by the difficulties in treating the disease, since the secondary tumors are hardly detectable and frequently resist to chemotherapeutic drugs (113, 114). Therefore, strategies that inhibit the spreading of the cancer cells or metastasis prevention for the patients in the high-risk group are recently of interest in the cancer research fields. Herein, we have reported for the first time that DF-A has an ability to sensitize anoikis, suppress migration, and inhibit anchorage-independent growth of lung cancer cells. Interestingly, the concentrations used caused on effect on the viability of adherent H460 cells and normal keratinocytes. This data supports the potentials of the compounds to be developed for the anti-metastasis approaches as it kills the detached cancer cells, while has minimal toxicity to the normal cells.

Anoikis refers to an apoptosis process that induces by the loss of cell adhesion. Basically, anoikis involves the disturbance in the balance of proteins in the Bcl-2 family and causes cells death through the activation of caspases (4). Overexpression of anti-apoptotic member of Bcl-2, such as Mcl-1 and Bcl-2 was demonstrated to render cells resist to anoikis in many studies (10, 76). In addition, the high level of Mcl-1 and Bcl-2 is frequently observed in highly metastasis cancer

cells (115, 116). These data have indicated the possibility that cancer cells may escape anoikis through an up-regulation of these anti-apoptotic proteins. Likewise, a number of studies shown that the increase in activated Akt level could render cells resist to many death stimuli (117). Also, several studies suggested the enhancement of activated Akt is associated with anoikis resistance in cancer cells (21, 89). Our investigations demonstrated that DF-A treatment resulted in a significant down-regulation of anti-apoptotic proteins Bcl-2 and activated Akt. Recently, Cav-1, a protein component of membrane structure caveolae, has received significant attentions from scientists as an important player in regulation of cancer cell behaviors. Although the role of Cav-1 in cancer biology remains controversial, recent studies suggested that Cav-1 inhibits cancer cell to anoikis and promoted metastasis (19-21). We and others have provided information that Cav-1 enhances metastatic potentials of lung cancer cells (19, 20, 118). Cav-1 was shown to increase invasion and migration (15, 16). Also, Cav-1 interacts and stabilized the level of anti-apoptotic Mcl-1 protein in the detached cancer cells (22). In the present study, we found the decrease of Cav-1 as early as 6 h after detachment concomitant with an increase of Bax, and believed that these proteins play a role in anoikis response in such condition.

It has been long known that the enhance ability of the cancer cells to migrate is an important potentiating factor of metastasis (23). The activation of several cellular pathways including FAK and Akt was shown to increase cell migration (29). Studies demonstrated that the phosphorylation of FAK at position Tyr 397 is important for cell migration (29, 119). Furthermore, effect of FAK in regulation of cell

motility was shown to be involved with its downstream Akt (30, 120). The function of FAK-Akt signal was shown to increase the active levels of Rho and Rac, which result in filopodia and lamellipodia formation and cell migration. As Rho-GTP regulates the polymerization of actin filaments toward forming filopodia (31), the inhibition of activated Rho may attenuate cell motility. We found that DF-A could decrease the activated FAK (pFAK) as well as active Rho in H460 cells. However, we only observed the insignificant decrease of active Rac in the cells treated with DF-A. This phenomenon was previously reported by others that in certain conditions Rac-GTP could not be detectable in migrating cells (121).

In summary, the present study reports a novel finding regarding the anti-metastasis effects of DF-A, a pure compound obtained from *Dendrobium falconeri* (Orchidaceae). DF-A was shown to sensitize lung cancer cells to death after detachment, decrease cell migration, and inhibit growth of the cells in anchorage-independent condition. Also, we provided the data about mechanisms of the compound in regulation of anoikis through the decrease of cellular activated Akt, Bcl-2, and Cav-1. Furthermore, we found that DF-A inhibits cancer cell migration through the suppression of activated FAK and Rho. As anoikis sensitization, inhibition of migration, and attenuation of growth in detached condition are recognized as possible strategies to overcome metastasis, such findings may benefit the development of this compound to be used for cancer treatment.