CHAPTER 5

DISCUSSIONS AND CONCLUSION

There is little information concerning the hormonal activity of vanilla bean. The nonsaponifiable fraction derived from the fresh green beans of *Vanilla siamensis* Rolfe ex Downie was assayed for the first time to detect their estrogenic activity. In this study, the estrogenic activity of *V. siamensis* plant extract was determined by a YES two-hybrid system harboring coactivators to establish similarity to the human estrogen gene regulation system. These compounds were able to exert an estrogenic activity *in vitro*, detected by induction of the β-galactosidase. The evaluation of estrogenic activity by YES method could be summarized that two YES systems, YES-hERα+hTIF2 and YES-hERβ+hSRC1, were able to response to 17 β-estradiol (E_2) as well as phytoestrogen composing in *V. siamensis* plant extract induced receptor depend transcription. *V. siamensis* plant extract was found to be YES-hERα+$hTIF_2$ but was not detected by YES-hERβ+$hSRC_1$. The advantage of the YES two-hybrid assay is a clear-cut estrogenic response initiated by ERα or ERβ, which is not possible to detect in the animal test that comprises both ERα and ERβ in some organs. Thus, YES is a useful assay to demonstrate differences of phytoestrogens with respect to ERβ over ERα.Whereas the magnitude of regulation was equivalent to E_2, the dose of *V. siamensis* plant extract needed for ERα activation or repression is greater than that of E_2 on a weight basis. However, because *V. siamensis* is a crude extract, the dose of the estrogenic components in the mixture in comparison with E2 is not known.

Since the initial identification of estrogen activity in plant extracts in 1926, numerous plants have been described with estrogenic properties (Farnsworth *et al.*, 1975). In the early 1980s, detection of plant estrogens was first demonstrated in human urine using gas chromatography-mass spectrometry and nuclear magnetic resonance which clearly showed that diet may be a source of estrogen (Axelson *et al.*, 1982; Setchell, 1985). The two-hybrid assay, which can be performed in yeast or mammalian cells, is an excellent system to analyze protein/protein interaction in vivo and can also be applied to estrogenic compounds (Nishikawa *et al*., 1999). One of the critical questions in the field of function in yeast is to what extent the behavior of receptor corresponds to its function in human cell. This study describes the effects of *V. siamensis* plant extract on the proliferation and differentiation of cultured hFOB cells. The hFOB cells are unique among the osteoblast models currently used in that they are conditionally immortalized normal human osteoblasts (Harris *et al*., 1995). Karyotype analysis on these cells revealed that they have only minor chromosomal translocations and deletions, which is in sharp contrast to the major chromosomal abnormalities that it can observed in MG-63 osteosarcoma cells by comparison. The time course of the expression of osteoprotegerin (OPG) in adult human osteoblast, it is a key factor regulating bone resorption and plays an important role in the episode of osteoporosis, and MG-63 osteosarcoma cells were similar to those alkaline phosphatase and osteocalcin. This OPG production in mature osteoblasts is greater than that in proliferating ones and the ability to inhibit osteoclast is greater in adult osteoblasts and OPG can be differentiated expressed by human osteoblast and MG-63 osteosarcoma cells at different culture stage, and reach its maximal expression at the stage of bone matrix maturity, E_2 exhibits positive effects on osteoblast to unregulated

OPG expression *in vitro* (Hou-*de et al.*, 2006). Thus, the hFOB cells make an excellent model system to study the function of osteoblasts and the effects of agents on osteoblasts *in vitro*.

We further examined its effects on the proliferation of hFOB cells and the bone mineralization process. The use of hFOB1.19 cells and the measurement of alizarin red-S staining reflect possible osteoblastic activity of a test compound, whereas the calcium nodule by cells is a marker for screening compounds acting as estrogen and cell growth promoters. The MTT test, on the other hand, tests the effect of a substance on cell viability and proliferation. *V. siamensis* plant extract induced mineralization of osteoblasts, and showed no effect on cell proliferation by the 11^{th} day of culture. These data indicate that *V. siamensis* plant extract exhibits the characteristic effects of a nature bone promoter compound, being an agonist in bone cells and may be attributed to the recruitment of tissue-specific ERα or ERβ coactivators or corepressors. Although the possible mechanism of this effect on hFOB1.19 cells growth is still not clear. Another plant compound, genistein, like estradiol and 4-ethoxymethylphenol, is influenced by certain ER modifiers such as cofactors, coactivators, and corepressors (Pearce *et al.*, 2003; Schwartz *et al*., 1998). Genistein induced osteoblast differentiation may be partly involved in estrogen action (Morris *et al*., 2006). Finally, vanillin, a phenolic aldehyde that is also reported to be a major degradation product of *V. siamensis* plant extract. Thus, the phenolic constituents of a methanolic extract of the stems *Sambucus sieboldiana* showed a suppressive effect on bone resorption both in vitro and *in vivo* and were examined for their inhibitory effect on bone resorption stimulated by PTH in neonatal mouse bone. Among them, vanillic acid, vanillin and coniferyl alcohol showed significant inhibitory effects on bone

resorption. Of the compounds examined; vanillic acid was found to have a significant inhibitory effect on the decrease of bone mineral density in ovariectomized (OVX) rat (Li *et al*., 1998).

Our observations indicate that the effect of *Vanilla siamensis* Rolfe ex Downie extract stimulates on cell maturation and osteoblast differentiation in hFOB cells. They may be beneficial in stimulating the osteoblastic activity resulting in bone formation. As a natural hormone, *V. siamensis* warrants further investigation.