CHAPTER IV

DISCUSSION AND CONCLUSION

Cancer is one of the major public health problems worldwide, and now has a very high mortality rate in Thailand. Cancer cells are typically classified by uncontrolled cell growth and cell division leading to a mass of unwanted cells called a tumor. Carcinogenesis is a complicated multi-step process. In 1863, Virchow hypothesized that the origin of cancer was at sites of chronic inflammation, in part based on his hypothesis that some classes of irritants, together with the tissue injury and ensuing inflammation they cause, enhance cell proliferation (129). The relationship between inflammation and cancer is more widely. Recent studies have unraveled molecular pathways linking inflammation and cancer. Inflammatory conditions can initiate and/or promote oncogenic transformation. There are many causes of chronic inflammation that augment the risk of developing cancer such as microbial infection (i.e. infection with *Helicobactor pyroli* is deal with gastric cancer and gastric mucosal lymphoma), autoimmune diseases (i.e. inflammatory bowel disease is concern with colon cancer) and inflammatory conditions of unknown origin (i.e. prostatitis is associated with prostate cancer) (130). An inflammatory microenvironment further supports tumor promotion and progression.

Cancer-associated inflammation is marked by the presence of specific inflammatory cells and inflammatory mediators (i.e. cytokines, chemokines and prostaglandins) regulating the growth, migration and differentiation of all cell types in the tumor microenvironment, including neoplastic cells, fibroblasts and endothelial

cells. Moreover, the recruitment of pro-inflammatory cytokines may also be counter for tumor development, and also may represent an attempt by the host to suppress tumor growth. The profile of cytokine/chemokines persisting at an inflammatory site is important in the development of chronic disease. It is now evident that pro-inflammatory cytokines have powerful effects on genetic and epigenetic changes in malignant cells.

In this study, the effect of IL-1β, TGF-β1 and TNF-α on expression of Maspin was explored in a variety of cancer cell lines. Preliminary result indicated that the cytokines affect only the cancer cell line, HeLa that has constitutively expressed Maspin. While IL-1β did not alter the level of Maspin, TGF-β1 clearly stimulated, but TNF-α slightly down-regulated the expression of Maspin in HeLa cells, respectively. The effects of both cytokines were dose dependent and on production of both mRNA and protein.

TGF-β1 is a member of a large family of cytokines that controls many aspects of cellular function, including cellular proliferation, differentiation, migration, apoptosis, adhesion and angiogenesis. One of the biological effects of TGF-β1 is the inhibition of proliferation of most normal epithelial cells using an autocrine mechanism of action, and this suggests a tumor suppressor role for TGF-β1. In late stages of tumor progression when tumor cells become resistant to growth inhibition by TGF-β1 due to inactivation of the TGF-β1 signaling pathway or aberrant regulation of the cell cycle, the role of TGF-β1 becomes one of tumor promotion. TGF-β1 can exert effects on tumor and stromal cells as well as alter the responsiveness of tumor cells to TGF-β1 to stimulate invasion, angiogenesis, and metastasis (87).

The effect of TGF-β1 on increased Maspin expression has been previously shown in MCF10A, transforming normal mammary epithelial cells (131). The result of this study has provided the first evident of TGF-β1 induced Maspin expression in cancerous cell. According to the study in MCF10A, the expression of Maspin requires wild-type p53 for TGF-β1 signaling of gene expression. In contrast, other study showed that normal human corneal stromal cells *in situ* express substantial amount of Maspin, but the expression was decreased by treatment of TGF-β1 upon the cultured corneal stromal cells (126). Thus, the effect of TGF-β1 on Maspin expression depends upon the type of cells. In the present study, although the expression of Maspin in HeLa was induced by TGF-β1, the increased level of Maspin was not sufficient for inhibiting the cancer cell invasion. This result was similar to the MCF10A study in which TGF-β1 could also stimulate the MCF10A invasion even though the Maspin expression had been induced by TGF-β1. However, we cannot rule out the biological of Maspin on cancer cell invasion because transfection of Maspin siRNA in TGF-β1-treated MCF10A cells led to a further increase of cancer cell invasiveness. The TGF-β1 signaling pathway in cancer cells have been shown to promote cancer invasion by up-regulating the expression of proteases such as MMP-2 and MMP-9(132). Therefore, it is likely that Maspin still plays its role on controlling cell invasion, but its action could not completely counteract with the effect of TGF-β1 on promoting cancer invasiveness.

Maspin was first identified as a putative suppressor of metastasis by virtue of its high level of expression in normal mammary epithelial cells as compared to its greatly reduced or absent expression in malignant cells (133). Although Maspin displays anti-

metastatic properties during mammary and prostate cancer development, its expression is maintained during ovarian, lung, and pancreatic carcinogenesis, indicating that Maspin-regulated metastatic potential is tissue specific. Localization of Maspin showed a nuclear distribution in the Maspin expressing cancer cells whereas most of the normal cells express Maspin in cytosol. Now, it is still unknown whether Maspin on different cellular location could play a different role, especially nuclear Maspin on cancer progression. Thus, the nuclear localization of Maspin in cancer cells treated with TGF-β1 would provide further information to support the role of TGF-β1 on tumor progression.

The pro-inflammatory cytokine TNF-α (tumor necrosis factor-α) is essential in all of the events in carcinogenesis, regulating a cascade of cytokines, chemokines, adhesions, MMPs and pro-angiogenic activities (72). Furthermore, TNF-α could induce cancer cell invasion (134), and the decreased Maspin expression by this cytokine may provide one of the explanations of the tumor-promoting effect of TNF-α. In this study, it is the first report of TNF-α –reduced Maspin expression in human cancer cell. Similar to this result, a previous study has showed that RANKL (receptor activator of NFκB ligand), which is a membrane-bound protein belonging to the TNF-α family of ligands, can down-regulate Maspin expression in prostate cancer cells (119). Furthermore, the invasion of prostate cancer cells was significantly increased upon loss of Maspin expression. Yet, in our study the effect of TNF-α did not effectively block the invasiveness of HeLa cells which is related to a small decrease on Maspin expression by TNF-α.

Loss of Maspin expression during tumor progression was demonstrated to result, in part, from absent Ets and Ap1 transactivation in combination with epigenetic silencing by methylation (38). Luo and colleagues (2007) now provide evidence that prior to silencing by methylation, the *maspin* promoter is transiently repressed by IKKα, In the study by Horswill et. al., the promoter of Maspin in corneal stromal cells *in situ* is hypomethylated, but become hypermethylated upon culture of the cells in serum medium. Thus, it is possible that the alteration of Maspin expression results from a change in methylation status of Maspin promoter following a cytokine signaling in cancer cells. However, in this study, either TGF-β1 or TNF-α affected the methylation status of Maspin promoter in HeLa cells, suggesting that the altered levels of Maspin expression are not mediated by this epigenetic mechanism. The response of HeLa to TGF-β1 signaling may be similar to those in MCF10A which involves Smad2/3 and p53 regulated expression of Maspin.

In conclusion, the effect of cytokine on Maspin expression was investigated in several human cancer cell lines. Only HeLa cells constitutively expressed Maspin while other cancer cell lines and normal fibroblast, MRC5 had no Maspin expression. IL-1β did not change the expression of Maspin in HeLa. After 24 h incubation, the level of Maspin transcript was markedly up-regulated by TGF-β1 at 0.1 ng/mL, and saturated at 10 ng/mL. The down-regulation of Maspin expression by TNF-α was apparently achieved at 1 and 10 ng/mL. Similar to the result of RT-PCR, TGF-β1 increased, but TNF-α decreased the level of Maspin transcript and protein in HeLa cells. In addition, the post-treatment of TNF-α could not reduce the effect of TGF-β1, but, TGF-β1 could overcome the activity of TNF-α, as the level of Maspin transcript

was similar to that induced byTGF-β1 alone. Surprisingly, the co-treatment of TGF-β1 and TNF-α resulted in the level of Maspin expression in the middle of those caused by each cytokine treatment. The result suggested that the induction of Maspin expression by TGF-β1 seemed to be antagonized by TNF-α. Dose response cytotoxicity profiles for TGF-β1 and TNF-α determined by Sulforhodamine B (SRB) assay indicated that TGF-β1 and TNF-α at 0.1 – 10 ng/mL were not cytotoxic to the HeLa cells. TGF-β1 significantly increased both cell migration and invasion by approximately 40 percent. Besides, TNF-α at 10 ng/mL slightly induce the migration, but the effect of TNF-α on cell invasion was not significantly different from the untreated control. Nonetheless, either TGF-β1 or TNF-α has no effect on CpG methylation sites of Maspin promoter. Finally, as the chronic inflammation is known to play a role on all steps of carcinognesis, cancer cells may continuously respond to various cytokine (TGF-β1 and TNF-α) signaling leading to progression such as invasion of cancer cells. One of the mechanisms by which cytokines regulate cancer invasion may involve regulation of Maspin expression. Finally, as a tumor suppressor, Maspin is nonetheless a promising predictive biomarker for progressive tumorigenesis, as well as molecular targets for prevention and treatment of cancer and metastasis.