CHAPTER V
CONCLUSIONS AND SUGGESTIONS

A solid tumor consists of both malignant cells and a number of nonmalignant stromal cell types, including endothelial cells, fibroblasts and largely infiltrating leukocytes. Cooperative interactions between these cells within the tumor microenvironment take part in creating the specific microenvironmental milieu for impact on tumor growth, progression, metastasis, and angiogenesis (Witz, 2008). A marked population of monocytes/macrophages is found mostly in tumors. These inflammatory cells have been implicated to play a pivotal role in the pro-malignancy function by producing growth and survival factors for inducing tumor progression (Allavena et al., 2008; Mantovani et al., 2008)

In this study, the role of monocyte/macrophage in blood and tissue of CCA patients in promoting tumor progression was emphasized. In Chapter II, the expansion of $CD14^{+}CD16^{+}$ monocytes in peripheral blood of CCA patients was evident. This expansion is related to tumor since the level of this monocyte subpopulation was reduced after tumor removal. With the presence of high amount of surface adhesion molecules found in these monocytes, it is likely that $CD14^{+}CD16^{+}$ monocytes may interact with endothelial cells and be actively recruited to the inflamed or malignant tissues. The monocytes from CCA patients also showed M2 characteristic (with high CD163, EREG, VEGFA, CXCL3 and low CXCL10 expression) and may be primed with tumor microenvironment to be M-2 macrophages which would facilitate progression of the CCA.

The study reported in Chapter III highlighted on the molecular signature of CCA associated blood transcriptome. By comparison blood transcriptomes derived from peripheral blood of CCA, BBD and healthy subjects, a differential gene profile specific to CCA was obtained, of which three candidate genes computed as "risk score" was innovated to discriminate between good and poor prognosis patients. The specified biological functions of the blood cells in CCA patients were speculated to involve in host immune response and cellular growth, angiogenesis, and proteolysis.

Expressions of the differentially expressed genes in CCA blood transcriptome were verified to be monocyte and leukocyte origins. This again emphasized on the significant and specific function of monocytes in CCA.

In Chapter IV, a relation of blood $CD14^{+}CD16^{+}$ monocytes and the recent blood derived macrophages (MAC387+ve cells) in CCA tissue was evident. High density of recent blood derived macrophages (MAC387+ve cells) in CCA tissues was located at leading edge of tumor, and perivascular area. These macrophages were shown to co-express uPA and MMP9 which played a supportive role in tumor metastasis. The association of these MAC387 macrophages and poor patients' outcome was demonstrated.

All together, this study has demonstrated the significance of inflammatory cells in CCA, namely monocytes/macrophages. The interaction between malignant cells and inflammatory cells, $CD14^{+}CD16^{+}$ monocytes in blood circulation, and MAC387+ve macrophages in tumor tissues in promoting tumor progression was emphasized. In addition, blood transcriptome can be used as tissue surrogate markers for monitoring or predicting the eventually outcome of the patients, for instance, the "risk score' as innovated in this study. The overall role and interaction of monocyte/macrophage in CCA concluded from this study is postulated as shown in the Figure 5.

For further studies, several independent sets of the differentially expressed genes in a large patient's population and with different set of subjects (including CCA, benign biliary tract disease and other gastrointestinal disorder) are suggested to explore for possible valuable indices related to CCA and CCA patients.

The interaction of immune component with tumor cells represents another case of microenvironmental components which could either restrain or promote tumor progression. It is now well established that the immune system functions as a double-edged sword in tumorigenesis and tumor progression and that protective antitumor immunity does indeed operate, whereas immune components may also function as pro-malignancy factors. Monocytes/macrophages have been implicated to play a pivotal role in pro-malignancy function during tumor developments. Until recently, CCA therapy trials targeted exclusively the tumor cells. The present study suggests that blocking tumor microenvironment interaction especially between tumor and

inflammatory cells that boost CCA progression may serve as alternative target for specific adjuvant treatment of CCA.

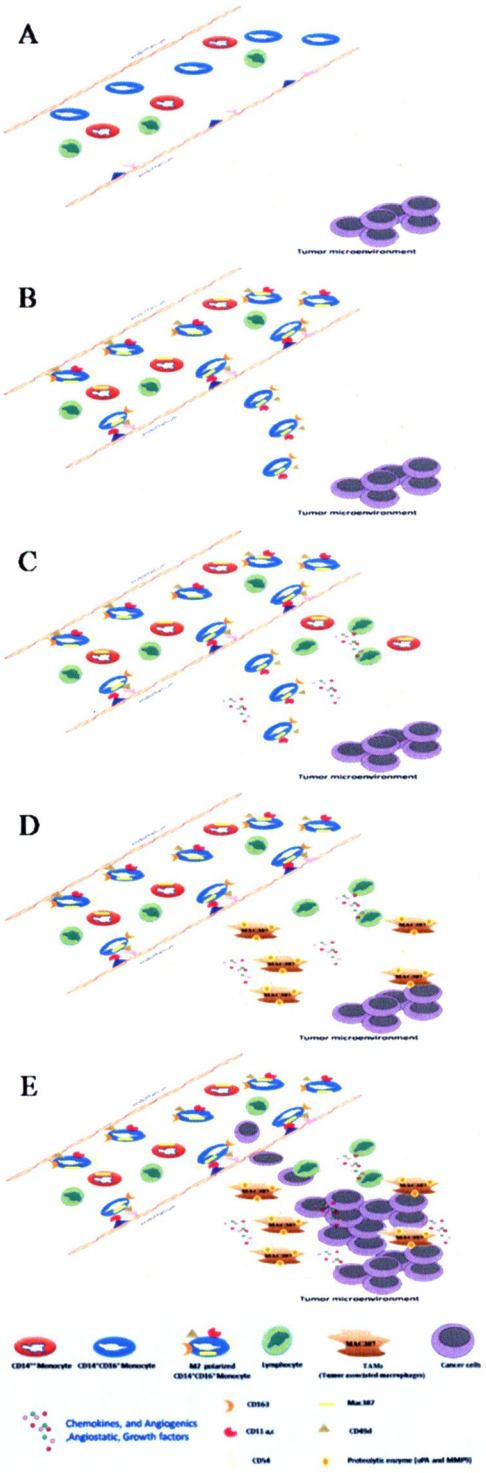

Figure 5 Overall role and interaction of monocyte/macrophage in CCA. (A) Expansion of $CD14^{+}CD16^{+}$ monocytes (blue) in peripheral blood of CCA patients was evident. (B) This monocyte subset is likely to interact with endothelial cells via high expression of surface adhesion molecules and actively recruited to the inflamed or malignant tissues with M2 characteristic including high CD163, EREG, VEGFA, CXCL3 and low CXCL10 expression. (C) Not only $CD14^{+}CD16^{+}$ monocyte was recruited into tumor microenvironment but lymphocytes also transmigrated. These infiltrating cells have been implicated to promote tumor progression via cellular growth, angiogenesis, proteolysis and others. (D) Monocytes ($CD14^{+}$ and $CD14^{+}CD16^{+}$) may serve as a subpopulation of infiltrating macrophages (MAC387+ve cells) in CCA tissue. (E) These infiltrating cells co-expressed protease enzymes (uPA and MMP9) which may play supportive role in CCA metastasis. (Subimerb C and Wongkham S).