# CHAPTER 4

# DISCUSSIONS

In the present study, we demonstrated that the *WT1* gene was expressed in various kinds of cancer cell lines such as breast cancer cell lines (MCF-7 and MDA-MB-468) and Prostate cancer cell lines (PC3 and LNCaP). The *WT1* gene was originally isolated as a tumor-suppressor gene responsible for Wilms' tumor, a kidney neoplasm of childhood (Call et al., 1990; Gessler et al., 1990). Subsequently, a growing body of evidences suggested that WT1 can act a survival factor in various types of human cancer. In breast cancer, Silberstein et al. (1997) have reported that WT1 immunostaining was decreased as compared with benign counterpart in the majority of breast cancer samples. . In contrast, other studies analyzing human breast cancer samples for *WT1* gene expression by RT-PCR demonstrated that the level *WT1* gene expression was higher in cancerous tissue as compared with benign mammary epitheliums (Loeb et al., 2001). Moreover, the prognostic value of WT1 gene expression was also observed in the study in which high levels of WT1 transcript as` detected by real time RT-PCR were found to be associated with adverse clinical outcome of breast cancer patients (Miyoshi et al., 2002). To further address this controversial issue, we employed RNA interference technology to specifically knock down *WT1* gene expression in breast cancer cell, MCF-7 to see whether this will inhibit the growth of the cell. We selected the synthetic siRNA (siRNA) for this purpose as the amount of siRNA can be accurately predetermined which can support the dose titration study. Our study used the mixed populations of $siRNA_{WT1}$ consisted of three siRNAs targeting three different regions of WT1 transcript as this strategy was believed to minimize the non-specific cytotoxic effect of siRNA. By using this technique, we were able to specifically knock down WT1 protein expression and inhibited growth of MCF-7 breast cancer cells. The kinetic of WT1 silencing by siRNA was also addressed by using the various concentrations of siRNA as well as time-course experiment. This study showed that $siRNA_{WT1}$ treatment to breast cancer cells induced growth inhibition in dose dependent manner in which the degree of growth inhibition was increasing when using higher dose of siRNA. If the assay was performed 72 h after transfection, the minimum effective dose was 25 nM. The degree of inhibition reached the plateau at 200 nM concentration. The silencing effect on WT1 protein expression also appeared to be dose-

dependent. This growth inhibitory effect was likely to be attributable to the WT1 protein down-regulation as no effect was observed in control cells in which no change in WT1 protein level was detected. This finding suggests that WT1 is indispensable for the survival of breast cancer cell and confirmed that siRNA can be used to effectively knock down WT1 gene therefore may be applied for the development of gene-targeted therapy for breast cancer. For the mechanistic point of view, this approach would be the powerful tool to dissect the molecular pathway involved in WT1-mediated carcinogenesis. We are currently conducting the gene expression analysis in breast cancer cells after transfected with $siRNA_{WT1}$ to identify the downstream molecules involved in this pathway. As WT1 gene was also found to be overexpressed in several human cancer cells whereas was not found to be expressed in most of benign cells, anti-WT1 approach by siRNA will be one of the ideal molecular-based treatment for certain human cancers. Further study in animal model would be required to evaluate the effectiveness of this measure.

Our data is in line with earlier study conducted by Zapata-Benacides et al (2002). This study showed that WT1 protein expression levels increased when proliferation of breast cancer cells was stimulated by 17β-estradiol, but decreased when inhibited by tamoxifen or all-trans-retinoic acid (ATRA) and that growth of breast cancer cells was inhibited by the WT1 antisense oligomer treatment which induced WT1 protein downregulation. This result suggested that one possible mechanism by which WT1 protein contributes to breast cancer proliferation is by regulating the levels of the cyclin D1 protein, which is known to be important to breast cancer progression (Musgrove et al., 1996). Recent study also addressed this molecular-mechanism underlying WT1 mediated oncogenesis pathway (Naama et al., 2005). In the experiment using transient transfection assay, IGF1R promoter activity was shown to be suppressed by all major spliced isoforms of WT1 in breast cancer cells whereas estrogen receptor-α (ER α) was shown to be able to stimulate this IGF1R promoter activity. Moreover, the physical interaction between WT1 and ER α were also demonstrated in that this interaction can abrogate the ER α -stimulating effect on IGF1R promoter. Based on this observation, *WT1* was suggested to act as a tumor suppressor gene. This finding is contradicted with previous functional study (Musgrove et al., 1996). However, recent study further addressed this controversial issue. Induced expression of different WT1 spliced isoformes in H16N-2 breast cancer cell which does not express endogenous WT1, induced different pattern of WT1-target gene, p21 expression as well as

cellular organization (Burwell et al., 2006). However, overexpression of WT1 (-Ex5/-KTS) induced upregulation of p21 and promoted the appearance of highly-organized acinar cellular aggregates. In contrast, overexpression of WT1 (+Ex5/+KTS) induced an epithelial-mesenchymal transition and redistribution of E-cadherin from the cell membrane to the cytoplasm albeit had no effect on p21 expression. This finding suggested that the biological function of WT1 in cancer cell is likely depended on the ratio of WT1 – spliced isoforms as well as the status of WT1 interactive proteins especially ER α or p53. This scenario suggests that the differential expression of WT1 spliced-isoforms should be evaluated before the maximum advantage of molecular-base therapy targeting WT1 can be obtained. The strategic design for inhibition of specific WT1 isoforms, which possess oncogenic property, will be highly beneficial for cancer treatment. Due to the fact that RNAi is known for its highly specificity and effectiveness for gene silencing, it would be the ideal tool for this purpose. Further study to find the best siRNA as well as the suitable gene transfer method will be the key for this succeed.