CHAPTER II

LITERATURE REVIEW

Cancer is increasingly known as a disease of uncontrolled cell growth. Therefore, key factors regulating cell growth and/or apoptosis may be targeted by specific biological agents to destroy cancer cells. In sensitive tumor cells, the molecular mechanism of anticancer drugs relies on the activation of apoptosis. Apoptosis can be induced by ligands binding to death receptors. One such death receptor is the TNF receptor (TNFR), activated by TNF-alpha. TNF-alpha triggers apoptosis through the activation of caspases in a wide variety of cells. The cellular effects of TNF-alpha occur through binding to its receptor (TNF-RI and TNF-RII), which leads to secondary signaling events. These signaling events start either an apoptotic or an anti-apoptotic pathway (Gupta S. 2002).

An anti-apoptosis effect of TNF-alpha has been demonstrated on many levels. Cell-line studies showed that TNF-alpha induced survival activity in ameloblastoma (AM-1 cell line) through Akt and MAPK pathways, while a prolonged treatment of 100 ng/ml TNF-alpha (24 h) to AM-1 cells resulted in apoptosis (Ferry S. 2006). In mouse model, tumors induced by exposing mice to azoxymethane (AOM) and dextran sulfate sodium (DSS) were reduced after administration of etanercept, a specific antagonist of TNF-alpha. The results suggested that blocking TNF-alpha can reverse carcinogenesis, probably by reducing the infiltration of inflammatory cells (Boryana K. P. 2008). Finally, *in vitro* observations showed that TNF-alpha was cytotoxic to L293 cells and cytostatic to Meth A sarcoma cells (Carswell EA. 1975). The transplantable methylcholanthrene-induced sarcoma model showed tumor regression with either direct intra-tumoral TNF injections or systemic intravenous TNF injections (Creasey AA. 1986). Animal

xenograft models have also shown that intra-tumoral injection of recombinant TNF can lead to tumor regression (Balkwill FR. 1986; Creasey AA. 1986).

To enhance the ability of TNF-alpha to kill tumors, a number of studies have examined the synergistic effects of TNF in combination with chemotherapy agents. Using human cervical carcinoma HeLa cell line, the cytotoxicity of TNF-alpha was greatly increased during *in vitro* hyperthermia (1-h heat-shock at 45 ^{o}C) (Marie-Francoise D. 1989). The combination of TNF and hyperthermia *in vivo* with transplantation of Meth A fibrosarcoma cells in mice also produced cures in 5 mice, compared to a partial response with TNF alone (Watanabe N. 1988). In cholangiocarcinoma cell lines, HuCCA-1 and HuCCA-1 Nu were found to be susceptible to apoptosis induced by TNF-alpha in presence of actinomycin D (Utaisincharoen P. 1999).

Although TNF-alpha was originally thought of as a selective antitumor agent, it is now known to have a paradoxical action as tumor promoter in many types of cancer. TNF-alpha is expressed in a range of human tumors and its protein and mRNA are present in tumor stages at higher than normal levels. In patients with advanced-stage nonsmall cell lung cancer (NSCLC), the serum level of TNF-alpha (8.8 pg/ml, n = 57), as determined by ELISA, was significantly higher than that of the control (4.77 pg/ml n =24) (p=0.029) (Duygu D. 2008). In chronic lymphocytic leukemia (CLL), the TNF-alpha plasma concentration was significantly increased in patients and that was negatively correlated with survival. Patients having a higher level of TNF-alpha had a shorter survival time (Alessandra F. 2002). Both mRNA expression and TNF protein have been found in human epithelial ovarian tumor cells and within the infiltrating macrophages. The p55 TNFR has also been detected within ovarian tumor cells and infiltrating

macrophages, but not in stromal macrophages, while TNFRI has only been found within the infiltrating macrophages (Naylor MS. 1993). Out of 49 biopsies taken from patients with breast cancer, 43 expressed TNF mRNA and protein, compared to 4/11 biopsies from patients with benign breast disease. The TNF was localized to tumor stroma and infiltrating macrophages. Furthermore, though the number of macrophages did not increase with tumor grade, the expression of TNF within the macrophages increased with tumor grade (Miles DW. 1994). A similar picture of increased production of TNF-alpha correlating with worse prognosis has been identified in patients with prostate cancer. In these patients, raised serum TNF-alpha levels were associated with a reduction in body mass index and other factors associated with cachexia, as well as a significantly increased mortality (Nakashima J. 1998). In addition, TNF-alpha has been shown to inhibit androgen receptor sensitivity, a poor prognostic indicator, and hence to induce androgen-independent proliferation in prostate cancer cell line (LNCaP cell line) (Mizokami A. 2000). In chronic B cell lymphocytic leukaemia, increased TNF-alpha levels were found at all stages, with a progressive increase in serum TNF-alpha levels in relation to the disease (Adami F. 1994).