CHAPTER I

INTRODUCTION

Tumor growth is angiogenesis dependent (Folkman, 1971; Folkman, 1972). Without angiogenesis, tumors remain small and do not threaten the life of the host (Folkman, 1996). Anti-angiogenic treatment strategies offer a number of compelling advantages over conventional cytotoxic cancer therapies that highlighted in recent reviews (Kerbel, 2000). Briefly, endothelial cells, the drug targets, are readily accessible to pharmacological agents. The ratio of tumor cells to endothelial cells has been estimated as 100:1(Folkman, 1996), leading to the expectation of an amplified antitumor effect when endothelial cells are successfully inhibited. Probably most importantly, in contrast to genetically unstable tumor cells, endothelial cells are genetically stable; making it unlikely that anti-angiogenic agent would induce drug resistance(Kerbel, 1997). Recently, several naturally occurring angiogenesis inhibitors have been identified.

Curcumin (diferuoylmethane) is a major yellow pigment in turmeric (*Curcuma longa*) and is widely used as a spice. Curcumin exhibits a variety of pharmacological effects, and has been reported to have anti-inflamatory (Huang *et al.*, 1991) and anti-tumor activities (Rao *et al.*, 1995). The anti-cancer properties of curcumin in animals have been demonstrated by the inhibition of tumor inhibition of tumor initiation induced by benz(a)pyrene and 7, 12 dimethyl benz(a)anthracene (Huang *et al.*, 1992) and tumor promotion induced by phorbol esters on mouse skin and on carcinogen-induced tumorigenesis in the stomach, duodenum and colon of mice (Huang *et al.*, 1988, 1994). Mechanisms by which curcumin causes cancer chemoprevention are thought to involve antioxidation (Jovanovic *et al.*, 1999), inhibition of kinases (Rao *et al.*, 1995), interference with the activity of transcription factors such as nuclear factor-κB and activator protein-1 (Huang *et al.*, 1991), and suppression of expression of the enzyme COX-2 (Plummer *et al.*, 1999). Furthermore, the genetic changes in carcinogenesis in several organs involve different genes, but curcumin is effective in preventing carcinogenesis in several organs. The possible explanation for this finding is that curcumin may inhibit angiogenesis. The anti-inflammatory properties of curcumin have recently been attributed, at least in

part, to suppression of prostaglandin (PG) synthesis (Huang, *et al.*, 1991; Rao *et al.*, 1995). The conversion of arachidonic acid to PGs is catalyzed by two isoenzymes: the constitutively expressed cyclooxygenase-1 (COX-1) and the inducible cyclooxygenase-2 (COX-2). COX-1 is expressed in most tissues that generate PGs during their normal physiological functions, and its expression does not fluctuate in response to stimuli (DeWitt and Smith, 1988;Yokoyama *et al.*, 1988). In contrast, COX-2 expression can be induced by various agents, including inflammatory cytokines, mitogens, reactive oxygen intermediates and many other tumor promoters (Smith *et al.*, 1996; Sheng *et al.*, 1997). The expression of COX-2 is significantly increased in various types of carcinoma, including hepatocellular carcinoma (Eberhart *et al.*, 1994; Sano *et al.*, 1995). The expression of COX-2 was correlated significantly with the depth of invasion, stage of disease and metastasis (lymph node and liver). It suggests that COX-2 is closely related to the invasion and metastasis, and COX-2 may be used as a possible biomarker.

Several studies have been reported that the overexpression of COX-2 in intestinal epithelium leads to increased carcinogenesis, metastatic potential and angiogenesis (Tsujii *et al.*, 1997; Tsujii *et al.*, 1998; Masferrer *et al.*, 2000). Masferrer *et al.* (2000) studied the correlation of COX-2 and angiogenesis of gastric cancer, and found COX-2 might regulate angiogenesis. Interestingly, COX-2 is also detected in non-cancerous cell immediately adjacent to tumor cells and in the angiogenic vasculature within tumors and in pre-existing blood vessels adjacent to tumors as well as the epithelial cells and some inflammatory cell (Koki *et al.*, 1999).

Recently, Zhang *et al.* (1999) have shown that curcumin inhibits the bile acid and phorbol ester-induced COX-2 expression in gastrointestinal cell lines. They also reported that curcumin can directly inhibit the COX-2 activity. This indicates that like other inhibitors of COX-2, curcumin can also inhibit the expression of COX-2. Since induction of COX-2 plays a role in angiogenesis, the activity of curcumin in inhibiting carcinogenesis in several organs may be mediated in part through angiogenesis inhibition.

An association between poor prognosis and increase in microvascular density of tumor has been reported in certain tumors (Sumiyoshi *et al.*, 2000; Ellis *et al.*, 2000;Jia *et al.*, 2000). This neoangiogenesis depends on the production of angiogenic factors by tumor cells and normal cells (Carmeliet and Jain, 2000; Teraoka *et al.*, 2001; Mancuso *et al.*, 2001). Numerous angiogenic factors have been described. Of these, vascular endothelial growth factor (VEGF) plays as a marker of the angiogenic process (Song *et al.*, 2002). VEGF is a multifunctional cytokine, and has direct relationship with angiogenesis. Elevations in VEGF levels have been detected in the serum of some cancer patients (Kondo *et al.*, 1994), and a correlation has been observed between VEGF expression and microvascular density (Toi *et al.*, 1994). A postoperative survey indicated that the relapse-free survival rate of patients with VEGF-poor tumors, suggesting that VEGF expression is associated with stimulation of angiogenesis. Moreover, there is compelling evidence that circulating VEGF levels are of prognostic significance in a variety of tumor types (Adams *et al.*, 2000; Bian *et al.*, 2000; Loncaster *et al.*, 2000; Mineta *et al.*, 2000; Stockhammer *et al.*, 2000; Yoshikawa *et al.*, 2000; Broll *et al.*, 2001; Hirai *et al.*, 2001). The factors that regulate VEGF expression in tumor and non-tumor cells have been elucidated (Gunsilius *et al.*, 2002; Xiong *et al.*, 2002; Tsuji *et al.*, 2002). Xiong *et al.* (2003) found that the expression of COX-2 was significantly correlated with the expression of VEGF. They demonstrated that COX-2 might be correlated indirectly with angiogenesis through an up-regulation of the expression of VEGF.

Although all of the above studies clearly demonstrate the potential chemopreventive activity of curcumin, there were no studies on the efficacy of this agent during the progression stage when the malignant lesions would have developed. In addition, there is still a few of experimental data for inhibitory effect of curcumin on tumor angiogenesis, especially, using carcinoma implanted nude mice model together with the visual ability of intravital fluorescence videomicroscopic technique. Therefore, the present study was designed to determine the effects of curcumin on angiogenesis in hepatocellular carcinoma cell (HepG2)-implanted nude mice and to study the effects of curcumin on HepG2 angiogenic biomarkers, COX-2 and VEGF levels.