CHAPTER VI

CONCLUSION

In the present study, using the intravital fluorescent microscope techniques, the effects of curcumin on angiogenesis in HepG2-implanted nude mice and biomarkers, COX-2 and VEGF levels were examined. The experimental data including NCD, capillary diameter, tissue perfusion, COX-2 and VEGF levels were determined for each group: Con, Con-cur300, Con-cur3,000, HepG2, and HepG2-Cur300, and HepG2-Cur3,000 at the experimental periods of 3, 7 and 14 days.

The significant findings could be summarized as follows;

1. HepG2 was able to grow by the model implanted dorsal skin-fold chamber on nude mice. Beside, the model was also allowed for evaluation of the progression of tumor angiogenesis.

2. By using intravital fluorescent microscopy, we could observe the development of HepG2-induced angiogenesis. 3 days after HepG2 inoculation, microvascular dilatation, tortuosity, hypermeability were early observed on before neovascularization begins. The tumor angiogenesis started to develop on day 7 after the inoculation of HepG2. The capillary networks were markedly increased on day 14 after inoculation.

3. There was a significant increase in the numbers of neocapillary density (NCD) with disorganization of the network in HepG2 groups. In particular this increment of neocapillary density was characterized as a time-dependent manner (NCD of 14 days > NCD of 7days). In our study, it is actually confirmed the fact that angiogenesis has already occurred on day 7 after HepG2 inoculation. A large number of neocapillaries was observed and corresponded with the increase in tumor-area tissue perfusion. It appeared that the neocapillary diameter have also been increased in these groups.

4. Western blot analysis data demonstrated that COX-2 strongly expressed in HepG2 groups within 3 days after tumor cells inoculation and maintained throughout during the experimental period. Moreover, the expression of COX-2 was significantly correlated with neocapillary density of HepG2 groups. These findings suggest that the overexpression of COX-2 may be functionally significant or acts as angiogenic biomarker in tumor angiogenesis, even in HepG2-implanted nude mice.

5. Serum VEGF increased in HepG2 groups with significantly raised on 7 and 14 days after HepG2 inoculation. A correlation between elevation of serum VEGF and NCD was lower than that between COX-2 and NCD. This suggests that COX-2 may be response to angiogenesic pathway first or upstream response mediator. This suggests that COX-2 may be response to angiogenesic pathway first or upstream response mediator.

6. Both the overexpression of COX-2 and elevation of serum VEGF were found in HepG2 groups. This suggests that the upregulation of both VEGF and COX-2 expression in the HepG2-implanted nude mice may be responsible for switching on angiogenesis.

7. No significant correlation was found between COX-2 expression and VEGF production. COX-2 and VEGF may co-modulate angiogenesis in different pathway.

8. Curcumin could inhibit angiogenesis which occurred in dorsal skin-fold chamber using HepG2-implanted nude mice model. Interestingly, our study has been shown that pathological angiogenesis features including microvascular dilatation, tortuosity, hypermeability are also attenuated by treatment with curcumin. Especially, the high dose of curcumin has not been indicated for its toxicity.

9. Curcumin has the potential to inhibit the expression of COX-2 at the dose of 300 mg/kgBW only on 3 days after treatment.

10. Increases of VEGF in the serum of HepG2 mice were also suppressed by curcumin.

11. Curcumin has anti-angiogenic activity *in vivo*. The activity of curcumin in inhibiting angiogenesis may be mediated in part through reduction of angiogenic stimulator or called biomarkers production.