CHAPTER 5

CONCLUSION

The DNA fragment encoding for the external domain of CD147 was inserted into the phagemid vector, pComb3HSS. The CD147Ex gene was fused to the gIII of the bacteriophage M13 and linked to the *Omp*A signal sequence. The recombinant phagemid vector was called pComb3H-CD147Ex. The pComb3H-CD147Ex was transformed into both *E. coli* XL-1 Blue and TG-1 strain. Transformed bacteria were separately cultured for phage display according to the standard protocol. The precipitated phage obtained from *E. coli* strain TG-1 was subsequently reinfected *E. coli* strain TG-1 for finding the phage concentration. The phage obtained from *E. coli* TG-1 strain is approximately 2.6×10^{10} t.u./ml. Direct ELISA was performed to find the quantity of the precipitated phage obtained from *E. coli* strain XL-1 Blue by using the known concentration of phage obtained from TG-1 strain as a standard. The concentration of precipitated phage from *E. coli* XL-1 Blue host is elucidated to be about 3.5×10^{9} t.u./ml. The CD147-linked gpIII was further subjected to sandwich ELISA. Six clones of CD147 mAbs were used to detect CD147 linked to phage particles obtained from both *E. coli* strains. Only four of six CD147 mAbs were reacted against CD147-Φ obtained from XL-1 Blue host, while all CD147 mAbs were reacted against CD147-Φ obtained from TG-1 host. It means that the *E. coli* strain TG-1 is more suitable to use for phage display CD147 than XL-1 Blue strain in term of CD147 conformation obtain.

Western immunoblotting demonstrated that the CD147-fused gpIII give the unique reactive band at the molecular weight about 38 kDa. The reactive band at about 38 kDa represents a 20 kDa of CD147Ex fused to 18 kDa of truncated gpIII. Epitope mapping of CD147 mAbs by competitive inhibition ELISA demonstrated that the epitope of some antibodies are overlapped with one another. The epitopes of M6-1B9 and M6-1E9 mAbs overlap each other but were not a neighbors of M6-2F9 epitope. M6-1B9, M6-1E9 and M6-2F9 obstructed binding of M6-1F3 mAbs to its epitope. However, mAb M6-1F3 did not influence the binding of any mAbs tested. This finding may be explained by allosteric effect. Epitope mapping by competitive inhibition ELISA is reliable and economical method. Certain clusters of mAb recognition areas were identified and will provide valuable information for the discovery of the ligand for CD147.

Since the CD147Ex displayed on phage particle is in nonglycosylated form, in assumption, all CD147 mAbs used in this study reacted with nonglycosylated epitopes. The CD147 displaying on phage particle is proper for applying in the experiment which purely nonglycosylated form is of need i.e. the effect on MMP-1 production from tumor cell. Apparently, if the nonglycosylated form can clearly show the reduction of MMP-1 expression level, it maybe applied for cancer therapy in the future.