Studies of *PTPN11*/SHP2 mutations in canine histiocytic sarcoma cells

Abstract of Doctoral Thesis

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Some canine cases of histiocytic sarcoma (HS) carry mutations in the src homology 2 domain-containing phosphatase 2 (SHP2) encoded by *PTPN11*. However, the precise mutational positions in SHP2 were not clear because entire coding nucleotide sequence of canine *PTPN11* has not been determined. In addition, functional role of the SHP2 mutations on structure and activity of SHP2 and growth of HS cells is unclear. SHP099 is an allosteric inhibitor of SHP2 that stabilizes SHP2 in a folded, auto-inhibited conformation. The purpose of this study was to make a foundation of SHP2-targeted therapy for canine HS. To investigate expression levels and mutation status of *PTPN11*/SHP2 in HS cell lines, firstly, entire coding nucleotide sequence of canine *PTPN11* was determined using cardiac cDNA isolated from healthy dog (registered with NCBI: GenBank accession number, MK_372881.1). Subsequently, expression levels and mutation status of *PTPN11*/SHP2 in HS cell lines were examined. All of six HS cell lines examined with western blot analysis were expressed SHP2 and four out of nine HS cell lines had mutations in *PTPN11*/SHP2 (p.Ala72Gly, CHS-1; p.Glu76Gln, CHS-3; p.Glu76Ala, CHS-6; p.Gly503Val, ROMA). Moreover, effects of mutations on the structures and the phosphatase activities of canine SHP2 were examined. Recombinant canine SHP2 harboring p.Ala72Gly, p.Glu76Gln and p.Glu76Ala showed constitutive phosphatase activities, while phosphatase activity

was not detectable in wild-type SHP2 and SHP2 harboring a p.Gly503Val mutation. The activities SHP2 harboring p.Ala72Gly, p.Glu76Gln and p.Glu76Ala were inhibited by SHP099. *In silico* analysis suggested that mutations p.Glu76Gln and p.Glu76Ala but not p.Ala72Gly and p.Gly503Val promote shifting of the SHP2 conformation from folded to open-active state. Furthermore, the growth inhibitory properties of SHP099 for HS cells were investigated *in vitro* and *in vivo*. Among six HS cell lines, SHP099 potently suppressed the growth of CHS-3 (p.Glu76Gln) and CHS-6 (p.Glu76Ala) cells. In contrast, other cell lines harboring SHP2 p.Ala72Gly, p,Gly503Val or wild-type had lower susceptibilities to SHP099. In HS xenograft mouse model using CHS-6 (p. Glu76Ala), SHP099 exhibited potent anti-tumor activity. In conclusion, p.Glu76Gln and p.Glu76Ala are activating mutations of SHP2 and play a pivotal role for survival/growth of HS cells carrying these mutations. Targeting p.Glu76Gln and p.Glu76Ala SHP2 with SHP099 may be a new therapeutic strategy for canine HS.