Functional analysis of human-adapted avian influenza viral polymerase

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論　文　概　要 (Thesis Abstract)

○ 論 文 題 目
(Theme)

Functional analysis of human-adapted avian influenza viral polymerase
(ヒト感染能を有する鳥インフルエンザウイルスポリメラーゼの機能解析)

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目　　的:
(Purpose)

Most avian influenza viruses do not replicate efficiently in human cells. Although several adaptive mutations in viral polymerase, which is composed of PB1, PB2, and PA subunits, of avian influenza viruses responsible for the host range have been reported, the detail is still unclear. In this study, I aimed to elucidate the molecular mechanism of mammalian adaptation of avian viral polymerase.

対象と方法:
(Material and method)

For this study, I used viral polymerases derived from influenza viruses A/duck/Pennsylvania/10218/84 (DkPen), A/WSN/33 (WSN), A/Hong Kong/483/1997 (HK483), and A/Hong Kong/486/1997 (HK486). Cell-based vRNP reconstitution assays and biochemical analyses using purified recombinant viral polymerases were performed to identify the polymerase activity, *in vitro* RNA synthesis activity, cap-snatching activity, and cap-binding activity.

結　　果:
(Result)

I found that avian viral polymerase from DkPen enhances the viral polymerase activity in mammalian cells by replacing the PA gene with that from human influenza virus WSN. Chimeric constructs between DkPen PA and WSN PA showed that the N-terminal endonuclease domain of WSN PA was essential for the mammalian adaptation of DkPen viral polymerase. I also found that the cap-snatching activity of purified DkPen viral polymerase was more than 5 times weaker than that of WSN *in vitro*. However, the cap-snatching activity of DkPen viral polymerase was hardly increased by replacing DkPen PA to WSN PA. In addition to this, it has been reported that a point mutation at Glu627 to Lys of avian PB2 subunit is crucial for the adaptation of avian influenza viruses to mammals, however the detail mechanism is unclear. PB2 subunit of HK486 possesses Glu at position 627, while that of HK483 contains Lys at this position. Thus, I examined whether the point mutation at position 627 is involved in the cap-snatching activity of viral polymerase. I found that the viral polymerase activity of HK483 was higher than that of HK486. When the mutations at PB2 627 position occurred, the polymerase activity of HK486 E627K mutant was significantly enhanced and that of HK483 K627E mutant was defected. I then found that the cap cleavage activity of HK486 polymerase is weaker than that of HK483. Further, the cap-snatching activity of HK486 E627K mutant was significantly increased and that of HK483 K627E mutant was impaired. However, the cap-binding activity performed by UV crosslinking of these two strains is not changed.

考　　察:
(Discussion)

Previous studies have identified the several adaptive mutations in PA among several subtypes of IAV with different genetic backgrounds. Some of the adaptive mutations in PA were identified in the N-terminal region of PA containing the endonuclease domain (a.a. position 1-197). In this study, I found that the replacement of DkPen PA to that from WSN enhances the viral polymerase activity of DkPen in the vRNP reconstitution assay. Chimeric constructs of DkPen PA and WSN PA also revealed that the peptide regions 184-370 and 540-716 from WSN PA cooperatively enhanced the viral polymerase activity of DkPen together with the N-terminal endonuclease domain from WSN PA. However, our biochemical experiments showed that the cap-snatching activity of DkPen viral polymerase is hardly increased by replacing DkPen PA to WSN PA. The viral polymerase activity observed in the vRNP reconstitution system is dependent on not only viral transcription but also viral genome replication. Thus, it is possible that the activity of viral genome replication is enhanced in the DkPen reassortant containing WSN PA. The adaptive mutation PB2 E627K is known to increase the viral polymerase activity in mammalian cells. HK483 and HK486 E627K vRNPs possessing PB2 627K position had the higher polymerase activity than HK486 and HK483 K627E. Furthermore, the biochemical analyses show PB2 627-domain is required for cap-snatching activity. However, recombinant polymerases containing the PB2 K627E mutant is able to carry out core polymerase functions similarly to viral polymerase consisting PB2 627K such as capped RNA primer-dependent transcription initiation, cap-independent *in vitro* RNA synthesis activity, and cap-binding activity, suggesting that the PB2 627-domain does not contribute to the core polymerase functions.

結　　論:
(Conclusion)

The cap-snatching activity of purified avian viral polymerase was weaker than that of human viral polymerase *in vitro*, although the viral RNA synthesis of avian viral polymerase was observed in avian cells, suggesting that avian cells should contain a positive avian host factor(s) that supports the avian viral polymerase activity. Therefore, additional studies examining avian host factors will be needed to understand the species adaptation of IAV. Furthermore, based on biochemical analyses, it is possible that the cap-snatching activity of avian viral polymerase is weaker than that of human-adapted viral polymerase due to the defective endonuclease activity of PA, which is possibly regulated by PB2 627-domain