Studies on the Molecular Pathogenesis and the Novel Disease-Associated Genes in Dogs with Inflammatory Colorectal Polyps

（犬の炎症性結直腸ポリープの分子病態および新規疾患関連遺伝子に関する研究）

Noriyuki Nagata

Summary

Inflammatory colorectal polyp (ICRP) in dogs is characterized by the presence of diffuse small polyps and/or large solitary polyps in the colorectal region. Although the pathogenesis of ICRP remains unclear, the effectiveness of immunosuppressive therapies indicated that immunological disturbances are associated with the pathogenesis of ICRP. The histopathological features of ICRP are well-studied, and hyperplastic goblet cells with abundant mucus and the infiltration of inflammatory cells, especially neutrophils and macrophages accompanied by the tissue granulation, are identified as the condition's distinct characteristics. However, the significance of these pathological findings in the pathogenesis of this disease is unclear. The most notable characteristic of ICRP in dogs is its apparent breed-specificity, with Miniature Dachshund (MD) accounting for more than 80% of the cases, strongly suggesting a genetic susceptibility in this disease. Despite the high suspicion of ICRP's genetic susceptibility in dogs, disease-associated genes have not been identified. Thus, the purpose of this study was to investigate the molecular pathogenesis of ICRP focusing on the histopathological features of this disease and explore the novel disease-associated genes involved in its pathogenesis.

In chapter 1, selected mucin gene expressions and goblet cell proportions were evaluated. There was no significant difference in the *MUC2* gene expression levels among groups. The expression level of *MUC5AC* in the polypoid lesions was significantly higher than that in the normal colonic mucosa and non-polypoid lesion. The percentage of goblet cells in the upper crypt regions was not significantly different between groups, while that in the lower crypt regions was significantly decreased in the ICRP polypoid lesions compared with that in the normal colonic mucosae.

In chapter 2, the activities of gelatinases (MMP-2 and MMP-9) in the colorectal mucosa were investigated. Activities of pro–MMP-2 and pro–MMP-9 were detected in most tissue samples regardless of group, whereas no activity of MMP-2 or MMP-9 was detected in the tissue samples from the control dogs. The activity of pro–MMP-2 was not significantly different among the 3 groups. On the other hand, the

activity of pro–MMP-9 in the inflamed tissue samples was significantly higher than that in the noninflamed samples or in the control tissue samples. The activity of MMP-2 and MMP-9 were significantly higher in the inflamed tissue samples than in the noninflamed tissue samples. Immunohistochemical analyses revealed that MMP-9 was abundantly expressed in the inflamed tissue samples from MDs with ICRP, especially in inflammatory cells such as neutrophils and macrophages. Expression of MMP-2 was detected in inflammatory cells and fibroblasts in the granulomatous tissue of the lesions.

In Chapter 3, the novel disease-associated genes for ICRP in dogs were explored using whole-exome sequencing. There were 31 missense variants in 25 genes, which were present in ICRP in MDs but not in beagles. Among these variants, one variant was shown to be rare and breed specific. The frequency of the variant, including homozygote and heterozygote, was significantly higher in ICRP-MDs than control-MDs which did not have the intestine pathology. The variant was not detected in other breed dogs. All homozygotes were present in ICRP-MDs. ICRP-MDs with the risk-alleles showed less intact molecules in the lesions compared to ICRP-MDs without the risk-alleles but no differences in the serum. Mechanistic analysis suggested that the amino acid substitutions induced by the risk-alleles reduce the stability of the molecules in the lesions of ICRP and reduce the level of intact molecules. Moreover, MMP-9 caused the reduction of the molecule *in vitro*. Overall, the results suggest that MMP-9 induces the reduction of the molecule with the risk-alleles in colons, which contributes to the high prevalence of ICRP in MDs with the risk-alleles.

In conclusion, the results of this study showed that changes of mucin gene expressions, goblet cell proportions, and MMP activities are present in the lesions of ICRP in dogs. Whole-exome sequencing revealed the rare and breed-specific variants, which was associated with the pathology. The results of this study provide insights into the pathogenesis of ICRP and the mechanisms of chronic inflammation.