News Release

Title

Elucidating a mechanism of species-specific aggregation of canine SOD1 with a degenerative myelopathy-linked mutation

Key Points

•E40K mutation induces aggregation in canine SOD1, but not human SOD1; However, the mechanism remains unknown.
•We found that the species-specific aggregation depended on a difference of the 117th amino acid residues, methionine and leucine in canines and humans, respectively.
•Crystal structural analysis revealed the instability of the hydrophobic core in canine SOD1 because of 117th methionine creating a cavity, but the cavity was filled by 117th leucine.
• Instability of canine SOD1 due to the cavity is the basis of species-specific aggregation of SOD1, and a clue for developing novel therapeutics for canine DM.

Summary

A research group led by Professor Koji Yamanaka, Lecturer Seiji Watanabe, and Graduate Student Kei Hashimoto of the Nagoya University, Research Institute of Environment Medicine, Tokai National Higher Education and Research System, has discovered the mechanism of species-specific aggregation of canine SOD1 with a degenerative myelopathy (DM)-linked mutation in collaboration with Research Fellow Hiroaki Kamishina of Gifu University and Professor Yoshiaki Furukawa of Keio University.

In DM, the E40K mutation, in which the 40th glutamate of the SOD1 protein is replaced with lysine, is thought to cause abnormal aggregation of the SOD1 protein, resulting in damage to motor neurons in the spinal cord of dogs. On the other hand, hereditary amyotrophic lateral sclerosis (ALS), a human neurological disease, also causes motor neuron damage due to abnormal aggregation of SOD1 protein, but the E40K mutation has no effect on human SOD1, suggesting that the aggregation of canine SOD1 caused by E40K mutation is species-specific. Therefore, this research group conducted research to elucidate the mechanism of species-specific aggregation of canine SOD1 caused by the E40K mutation, and discovered that canine SOD1 is less

stable and aggregates more easily than human SOD1, because canine SOD1 intrinsically has a ”gap” in the highly hydrophobic region inside the protein. By manipulating the presence or absence of this ”gap,” they also reproduced the species-specific aggregation of SOD1 protein caused by the E40K mutation. This indicates that the inherent vulnerability of canine SOD1 derived from the ”gap” is the cause of the species-specific aggregation by the E40K mutation. The results of this research are expected to lead to the development of novel therapies for DM in the future. This work was published online in the *Journal of Biological Chemistry* on May 6, 2023.

Research Background

Canine degenerative myelopathy (DM) is a fatal neurodegenerative disease in which motor neurons in the spinal cord are injured, resulting in progressive paralysis of muscles (Awano et al. 2009 *PNAS*), and the replacement of glutamic acid at position 40 of the Cu/Zn superoxide dismutase (SOD1) protein encoded by the *SOD1* gene with lysine (E40K mutation) is thought to be a primary cause.

ALS and DM caused by mutations in the *SOD1* gene share many clinical and pathological similarities. For this reason, DM has been recognized as a spontaneous disease model of ALS. Previous studies have shown that in familial ALS, intracellular aggregation of SOD1 protein induces motor neuron degeneration. On the other hand, canine SOD1 also aggregates due to E40K mutation, but there are no E40K mutation that causes ALS, and introduction of E40K mutation into human SOD1 does not cause aggregation (Crisp et al. 2013 *Exp Neurol*), suggesting that the aggregation of canine SOD1 by the E40K mutation is species-specific. If the E40K mutation causes aggregation through a mechanism unique to canine SOD1 that is completely different from that of human SOD1, then it is necessary to develop a suitable treatment for the E40K-linked DM. Therefore, the aim of this study was to elucidate the molecular mechanism of the species-specific aggregation of canine SOD1 caused by the E40K mutation.

Research Results

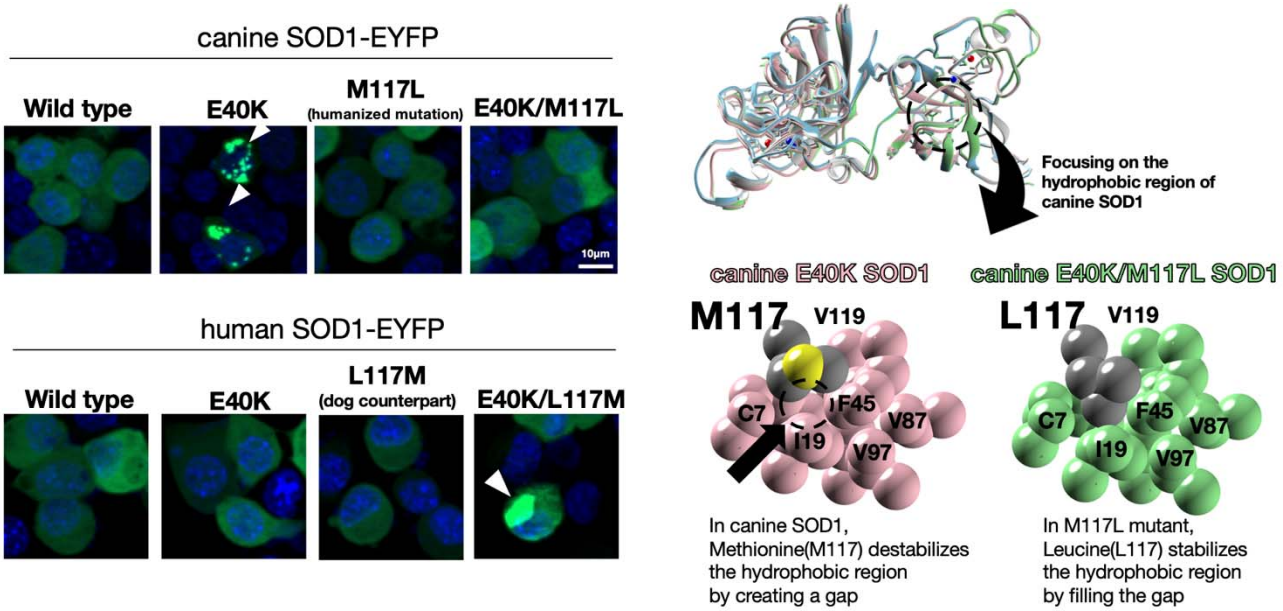

Figure. Overview of this study

Canine and human SOD1s have 20% differences in the amino acid sequences, and this research group hypothesized that amino acid residues that differ between these species may be responsible for the species-specific aggregation and aimed to identify them. Chimeric SOD1s, in which a part of canine SOD1 was replaced with human SOD1, were generated and verified as to whether the proteins aggregated, revealing that the 117th amino acid residue (methionine in canine and leucine in human) is important for the species-specific aggregation. Replacement of methionine at position 117 in canine SOD1 with leucine, the human counterpart, markedly suppressed the E40K mutation-dependent aggregation of canine SOD1 (Figure left upper panel). Conversely, replacement of leucine at position 117 with methionine in human SOD1 induced the E40K mutation-dependent aggregation of human SOD1 (Figure left lower panel).

The research group subsequently examined how this 117th amino acid residue is important for the species-specific aggregation of canine SOD1. Previous studies have shown that many aggregated proteins are unstable in structure, and their protein structures are easily destroyed by heating. In fact, canine SOD1 was found to be less stable for heat than human SOD1, even in the wild-type. This suggests that canine SOD1 is intrinsically more prone to aggregation than human SOD1. Interestingly, when the 117th methionine identified in this study was replaced with the human counterpart, leucine, the thermal stability of canine SOD1 was dramatically improved, whereas the thermal stability of human SOD1 was inversely reduced when the 117th leucine in human SOD1 was replaced with the methionine. Moreover, the replacement of

methionine with leucine reduced cytotoxicity of canine SOD1 with the E40K mutation. These results suggest that the 117th amino acid residue, leucine, stabilizes the structure of both canine and human SOD1 proteins and is important for suppressing aggregation and cytotoxicity caused by the E40K mutation.

Then, why is SOD1 stabilized by the 117th leucine? To answer this question, the research group further analyzed the structure of canine SOD1 by X-ray crystallography. As a result, methionine created a ”gap” in the hydrophobic region located inside canine SOD1 (Figure right). Furthermore, replacement of the 117th methionine with leucine filled this ”gap”. These results suggest that, unlike human SOD1, canine SOD1 is structurally vulnerable due to the ”gap” created by the 117th methionine, which is the cause of the species-specific aggregation by the E40K mutation.

Research Summary and Future Perspective

We have elucidated the key mechanism of E40K-dependent species-specific aggregation of canine SOD1. The results of this study indicate that canine SOD1 has the intrinsic vulnerability of the hydrophobic region derived from 117th methionine which forms a ”gap” there. Stabilization of this hydrophobic region may suppress abnormal aggregation of canine SOD1 and inhibit the onset and progression of DM.

Publication

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