（第３号様式）

学　位　論　文　要　旨

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論　文　名　ヒト death domain superfamily インタラクトームの網羅的研究と自己免疫疾患への応用

学位論文要旨

Human death domain superfamily is one of the largest and most studied domain superfamilies. It comprises 4 subfamilies: death domain (DD) subfamily, death effector domain (DED) subfamily, the caspase recruitment domain (CARD) subfamily and the pyrin domain (PYD) subfamily. Death domain superfamily proteins (DDSPs) are characterized by containing death-fold domains (DFDs). They are involved in various signal pathways of apoptosis and inflammation by assembling oligomeric complexes via homotypic bindings and inducing caspase and/or kinase activation. On the other hand, genetic mutations in DFD-containing proteins often cause variety of immunodeficiency and autoinflammatory diseases. For example, FAS interacts with fas-associated death domain (FADD) through DD and recruits pro-caspase-8 through its two DEDs and DED of FADD for forming the death inducing signaling complex (DISC).

Although these evidences have been accumulating, there is no mutations found in responsible genes, even in phenotypically diagnosed cases of these diseases. This fact prompts us to comprehensively analyze the interactions between all the DDSPs, which may provide clues to decipher pathways and factors that are associated with the immunodeficiency and autoinflammatory diseases.

In this study, we for the first time report the DDSP domain interactome analysis. The greatest difficulties in investigation of the interactome of DFDs were protein synthesis and domain-domain interaction analysis. We successfully synthesized and 116 FALG-tagged and 116 biotin-tagged domains of

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DDSPs using wheat germ cell-free protein synthesis system and performed comprehensive domain-domain interaction assay using ALPHA. Totally 13924 pair reaction was conducted in duplicate. The interactome assay data show that positive interactions come from 30% self-interacting pairs and 5.1% non-self-interacting ones, indicating that more positive interactions tend to be from the self-interacting pairs than non-self-interacting ones. Even though there are two domains containing heterogeneous DFDs, all the self-interacting pairs self-interact via homotypic bindings.

The results of AlphaScreen assay of domain-domain interactions turned out to be notably reliable and trustable, proved by MA plot, Bubble chart, immunoprecipitation assay and some previous reports. In addition, novel possible interaction candidates were found. Nine novel candidates from double-sided interacting pairs are worth believing to be reliable, because many of the double-sided interacting pairs are consistent with previous reports. As to the one-sided interacting pairs, which are supposed to be less reliable than those with few previous interaction reports, they also should be worth further testing, because a heterogeneous pair interaction was confirmed in the immunoprecipitation assay.

We believe the interactome investigation of DDSPs in this study enables progresses in the field of DDSP-related rare diseases, especially in immunodeficiency and autoinflammatory diseases. This work may shed light on the future research of, particularly identification of pharmaceutical targets for treatment and drug discovery.

キーワード（３～５）	human death domain superfamily, interactome, immunodeficiency and autoinflammatory disease, cell-free protein synthesis, amplified luminescence proximity assay