氏名（本籍）	工藤　生　（秋田県）
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学位論文題目（英文）	アリル炭化水素受容体とHSP90シャペロン複合体機能の解析に関する研究 (Studies on analysis of aryl hydrocarbon recepter (AhR) and HSP90 chaperone complex function.)
論文審査委員	（主査）教授　伊藤　英晃 （副査）教授　尾髙　雅文 （副査）教授　涌井　秀樹 （副査）教授　疋田　正喜

論文内容の要旨

The maintenance of proteins is essential for cells or individuals to survive. Synthesized proteins are natively folded and become functional. However, some of them are aggregated or misfolded from the effect of the temperature, pH, chemical agents and so on. Those misfolded proteins are helped by molecular chaperones which provide a “cage” to refold to the proper conformation. If proteins are not properly refolded or removed, they sometimes accumulate and cause various protein-aggregation diseases like Alzheimer's disease, Parkinson's disease and age-associated disorders.

Aryl hydrocarbon receptor (AhR) is a member of the nuclear receptor superfamily, it has the basic-helix-loop-helix/Per-Arnt-Sim (bHLH/PAS) domain for the dimerization and ligand binding, and also known to be a dioxin receptor. AhR is thought to be stabilized by the molecular chaperon heat shock protein 90 (HSP90), its co-chaperone p23, and hepatitis B virus X-associated protein 2 (XAP2) in the cytoplasm as a complex. In the presence of AhR ligands such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), ß-naphthoflavone (ß-NF), and 3-methylcholanthrene (3MC), AhR binds to ligands, translocates into the nucleus, then act as a transcription factor to promote cytochrome P450 1A1 (CYP1A1), a xenobiotic metabolizing enzyme.

Recently, interactions of AhR with ligands and HSP90 have been gradually revealed, but it is not fully understood in terms of the physiological potential of AhR or reasons for the existence of components of AhR-HSP90 complex for the AhR activity; particularly in the difference among each AhR ligand-toxic or non-toxic-.

In this study, I report the binding properties of AhR domains to HSP90 using purified proteins, interactions with AhR ligands, and intracellular functions of the AhR-HSP90 complex. We cloned AhR-PAS, AhR-bHLH, HSP90-N/M/C/ΔN/ΔM/ΔC domains, p23 and XAP2 into several expression vectors and purified by using affinity columns. As a result, we observed that the GST-tagged AhR-bHLH domain could bind to xenobiotic responsible element (XRE) by using a XRE-Sepharose affinity column and gel-shift assay. And previously, we showed that the AhR-PAS domain has abilities to bind ligands and HSP90, so we investigated whether the AhR-bHLH domain could also bind to them.Using purified HSP90 each domain and deletion mutants, p23 and XAP2, we confirmed that the AhR-bHLH domain also has an ability to bind to the HSP9-N domain as same as the AhR-PAS domain, furthermore, the AhR-bHLH domain interacts with p23 and XAP2 via full length of HSP90 to make a complex. These interactions among the AhR-bHLH domain and HSP90, p23 or XAP2 are not affected in the presence or absence of ATP or a HSP90 inhibitor 17-DMAG. We also examined whether the AhR-HSP90 complex translocated into the nucleus in the presence of 3MC by immunofluorescence in cervical cancer HeLa cells, and successfully confirmed AhR, HSP90, p23 and XAP2 translocated all together.

This study also includes an insight about the effect of 1,4-dihydroxy-2-naphthoic acid (DHNA), a novel non-toxic ligand of AhR derived from *Propionibacterium freudenreichii*. ET-3 isolated from Swiss-type cheese. Recently, some studies about AhR ligands indicated that AhR activation has the immunostimulating effect and inhibits inflammation in the gut.

So, we examined AhR activation by DHNA and the immunostimulating effect via AhR activation in human cell lines. As compared with 3MC, DHNA was not toxic for the cell viability and slightly increased cell population, induction period of CYP1A1 was shorter than 3MC. Then We observed the translocation of AhR-HSP90 complex by immunofluorescence and *in situ* proximity ligation assay (PLA), p23 did not translocate into the nucleus by DHNA, which indicates that DHNA as non-toxic ligand is recognized by different components of AhR-HSP90 complex, and this result suggests AhR can subtly change its conformation for a diversity to accept several ligands. And at present, we found that 3MC and DHNA induced IL-12 and IFN-γ in human epithelial Caco-2 cells. This immunostimulating effect needs further investigation using co-culture

system in Caco-2 cells and immune cells.

In conclusion, this study revealed AhR-bHLH domain function and how AhR interacts with HSP90, p23 and XAP2 *in vitro*. And also, this will be a basis for understanding of differences between toxic and non-toxic ligands of AhR *in vivo* and for further application of treatments and health maintenance by using DHNA.

論文審査結果の要旨

The maintenance of proteins is essential for cells or individuals to survive. Synthesized proteins are natively folded and become functional. However, some of them are aggregated or misfolded from the effect of the temperature, pH, chemical agents and so on. Those misfolded proteins are helped by molecular chaperones which provide a "cage" to refold to the proper conformation. If proteins are not properly refolded or removed, they sometimes accumulate and cause various protein-aggregation diseases like Alzheimer's disease, Parkinson's disease and age-associated disorders.

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