学 位 論 文 内 容 の 要 旨

博士の専攻分野の名称　博士（生命科学）　氏 名 林 暁智

学 位 論 文 題 名

In silico and *in vitro* investigations on the molecular mechanism of steroid hormone response of elephant shark, *Callorhinchus milii*, progesterone receptor
（*In silico* 及び *in vitro* 解析を用いたゾウギンザメのプロゲステロン受容体のホルモン応答性の分子基盤解明）

Progesterone receptor (PR) is a ligand-dependent transcriptional factor which plays important roles in the reproductive biology of vertebrates. A recent study on genome of a cartilaginous fish, elephant shark, has provided an interesting insight into the evolution of gnathostome, which makes this fish an ideal model for the comparative analysis and evolutionary study of steroid hormone receptors (SHRs).

In the first chapter, I analyzed the amino acid sequence of elephant shark PR which is cloned in our laboratory and compared with PRs of other vertebrates. Results showed that the DNA-binding domain and ligand-binding domain are highly conserved during evolution. The gene expression analysis of synthetic enzymes of steroid hormones indicates the possibility that progesterone, 17OH-progesterone and 5α-dihydro-progesterone is the physiological ligand for elephant shark PR.

The ligand responses of full-length and truncated elephant shark PR were investigated and compared with human and zebrafish PRs. The results showed that full-length elephant shark PR can be stimulated by more steroids than human and zebrafish. Elephant shark and human PRs still showed transactivation after the removal of A/B domain while truncated zebrafish PR almost lost the response ability to steroids. Chapter 1 provides an insight into the endocrine system of elephant shark as well as the ligand-dependent transcriptional function of elephant shark PR.

In chapter 2, the effects of mifepristone (RU486), a widely used clinical antagonist of human PR, was examined on elephant shark PR. Results showed that RU486 did not inhibit the progesterone-induced activation of elephant shark PR. Gly-722 in human PR corresponding to Cys-528 in elephant shark PR, which is on the helix 3, is considered essential for the antagonistic effects of RU486. Mutant PRs with cysteine/glycine substitution on helix 3 were constructed to confirm the relevance of this amino acid to the antagonistic effect of RU486 after mutation was recorded. A decline in steroid response of elephant shark PR-Gly528 by 11-deoxycortisol and an increase in activation of human PR-Cys722 were observed. To understand the molecular mechanism of this phenomenon, I investigated the ligand-receptor interactions between PR and 11-deoxycortisol using *in silico* methods including docking simulation and molecular dynamic (MD) simulation. The simulations provided a possibility that the interaction with cysteine on helix 3 and methionine on helix 7, Met-607 in elephant shark PR corresponding to Met-801 in human PR, is crucial for 11-deoxycortisol binding stability of PR. To confirm the role of the methionine residue, mutant PRs with methionine to glycine mutation on helix 7 were constructed and their steroid-induced activation were tested. The mutant PRs did not show any steroid response, which indicated the loss of interaction between methionine on helix 7 can lead to the loss of steroid hormone response for PR.

In order to further understand the actual role of the methionine on helix 7 to PR-11-deoxycortisol interaction, methionine to leucine mutant PRs were constructed and evaluated by *in silico* analysis. The simulations indicated that methionine to leucine mutation on helix 7 is comparatively stable than methionine to glycine mutation, suggesting that the steroid-induced activation of PRs are maintained after mutation. Chapter 2 provides us with some further understanding into the role of the interaction between ligand and helices in the ligand-binding stability of PR, which has never been described previously.