

## 2013 Abstract of thesis for doctor degree

# **Siah2, which causes a small eye phenotype by degradation of PHD, regulates the level of Nrf2 escaped from Keap1**

**Kwansei Gakuin University, Graduate School of Bioscience,  
Imaoka laboratory, Kazunobu Baba**

Sina was found as the factor which causes a small eye phenotype of *Drosophila*. *Xenopus* Siah2 (xSiah2) was isolated, and it was overexpressed during the development of *Xenopus laevis*, resulting in the formation of a small eye phenotype. The small eyes are characterized by a reduced size of the lens. Two highly conserved human homologs of *Drosophila* Sina, termed Siah1 and Siah2, were characterized. Siah2 regulates the stability of prolyl hydroxylase domain (PHD), with a concomitant effect on hypoxia inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ) availability in the hypoxia response pathway. Hypoxia is an important physiological condition during embryonic development. The hypoxia response pathway contributes to eye development during the embryonic development of *Xenopus laevis*, however, the role of Siah2 in eye development of *Xenopus laevis* embryos remains unknown. In Chapter I, the role of Siah2-mediated hypoxia response pathway in eye development was characterized. *Xenopus* Siah2 (xSiah2) mRNA was detected in lens tissue and xSiah2 overexpression caused a thickened lens placode, leading to loss of the optic lens. xSiah2 overexpression increased *Xenopus* HIF-1 $\alpha$  (xHIF-1 $\alpha$ ) accumulation. xHIF-1 $\alpha$  degeneration with HIF-1 $\alpha$  inhibitor restored the optical abnormality, suggesting that the xSiah2-induced HIF-1 $\alpha$  abundance causes abnormal lens phenotype. Additionally, Nrf2 protein was suppressed by hypoxia, and the suppression of Nrf2 under hypoxia was restored by the proteasome inhibitor, suggesting that some unidentified hypoxia-activated E3 ubiquitin ligase may be involved in the degradation of Nrf2. In Chapter II, I described the mechanism related to degradation of Nrf2 under hypoxia. Inhibition or knockdown of Siah2 prevented the suppression of Nrf2. Moreover, Siah2 interacted with Nrf2 through a binding motif, suggesting that Siah2 contributes to the suppression of Nrf2. Some cytosolic kinases also play important roles in Nrf2 regulation. Though PKC phosphorylates serine residues of Nrf2 during hypoxia, knockdown of Siah2 rescued hypoxic decreases in an Nrf2 mutant that mimicked phosphorylation at serine 40 or lacked this phosphorylation site, suggesting that Siah2 contributes to the degradation of Nrf2 irrespective of its phosphorylation status.

