Study on Stability and Regulation of Tetramer Formation in Tumor Suppressor Protein p53

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Chapter 1 General Introduction and Aim of This Study

The tumor suppressor protein p53 is the most important proteins for maintaining a biological activity in the cell, which is activated in response to several cellular stresses such as DNA damage, hypoxia, oxdative stress, and nutrient depletion. In order to activate the p53 function, it is essential for the p53 to form a tetramer. Tetramer formation of p53 is in equilibrium between monomer and tetramer, and it depends on concentration and temperature. Activation of p53 induces cell cycle arrest, apoptosis, cell differentiation, and autophagy. In this way, p53 related to a lot of signal pathway, and work as the core of signal transduction. Until now, although it had been reported lots of studies on p53, the regulatory mechanism is not completely understood, because of the complexity and diversity of p53 functions derived from lots of target genes and post-translational modifications.

In this study, I was aiming to reveal a novel regulatory mechanism of tetramer formation and to understand the significance of regulating the amount of p53 tetramer in the cell. I investigated the effect of arginine methylation for stability of tetrameric p53. And I also quantitatively analyzed the correlation between thermodynamic stability of p53 tetramerzation domain *in vitro* and expression level *in vivo*. Finally, in order to control the p53 function via heterotetramer formation, I developed a novel peptide inhibitor against the transcriptional activity of p53.

Chapter 2 Effect of Tetramer Formation by Arginine Methylation

In response to the cellular stresses, the tumor suppressor protein p53 is activated, tetramerized and induces cell cycle arrest, apoptosis and so on. Tetramer formation of p53 is essential for its function, and posttranslational modifications also contribute to the activity of p53 function. Recently, it was reported that three Arg residues in the tetramerization domain, Arg333, Arg335, and Arg337, were methylated by the protein arginine methyltransferase, PRMT5. In this study, I synthesized and analyzed eleven p53Tet peptide analogues with mono- or di-methylated-Arg at residues 333, 335, and/or 337 in order to clarify the effects of arginine methylation on the tetramer formation. *In vitro* methylation assay revealed the methylation cascade started at Arg335. The analysis of denaturation and isothermal titration calorimetry showed that all p53Tet peptide analogues formed the wild type-like homo-tetramers, however, the tetrameric structures were significantly destabilized. Methylation at the Arg337 residue induced much higher destabilization of the tetramer formation, compared with Arg333 and Arg335. This destabilization should be due to disruption of a hydrogen bonding between Arg337 and Asp 354 by mono- or di-methylation of Arginine residue. My study suggested that PRMT5 decreases the tetrameric stability of p53, resulting in the change of concentration in p53 tetramers, and this regulates the target gene specific transactivation.

Chapter 3 Correlation between Protein Expression Level in *Escherichia Coli* and Thermodynamic Stability

The point mutations in tetramerization domain of p53 induce destabilization of tetrameric structure and inactivation of cellular functions of p53. However the effect for structural stability of p53 on the expression level of p53 protein in the cell is still unknown. Protein expression using *E. coli* is a common and important method for recombinant protein preparation. Herein, I quantitatively analyzed the correlation between protein expression in *E. coli* and thermodynamic structure stability *in vitro* using tetramerization domains of various mutant p53s. I found a strong positive relationship between the expression level and the thermodynamic stability. These results suggested that destabilization of tetrameric structure of p53 could reduce the amount of

endogenous p53 protein in the cell and that the minimum thermodynamic stability of a protein should be required for substantial protein expression in bacterial cells.

Chapter 4 Inhibition of Tumor Suppressor Protein p53-dependent Transcription by Hetero-tetramerization

It is reported that a p53 suppresses or induces various cell differentiation and reprograming such as iPS cell generation in addition to regulation of cell cycle and apoptosis. However, constitutive suppression of p53 activity causes the cell to be potentially tumorigenic. Therefore, the transient inhibition of p53 is extremely valuable for cellular engineering. In this chapter, I report transient inhibition of p53-dependent transcription using a novel peptide of p53 tetramerization domain conjugated with cell penetrating and nuclear localization sequences. The peptide was efficiently introduced into cells and inhibited p21 expression via hetero-tetramerization with endogenous p53 protein.

Chapter 5 Conclusion

Tumor suppressor protein p53 plays an important role in maintaining genomic integrity in a normal cell. Because of tumor suppressor functions of p53, a half of malignant tumors in human have a mutation in p53. Moreover, it had been reported that p53 is also involved to cell differentiation, reprograming and metabolism.

In this study, I clearly showed that the methylation of Arg333, Arg335, and Arg337 by PRMT5 destabilized the tetramer formation of p53. It was suggested that p53 is often regulated negatively according to the situation. I also revealed that structural stability of oligomerization is related to the expression level of the protein in *E. coli.* These results have showed that the destabilization of p53 structure affects not only their function but also the expression level in the cell. It was suggested that threshold of destabilization is so severe that destabilization might result in the excessive degradation of p53 protein. Finally, I developed the transient inhibitor of the p53 transcriptional activity called p53Tet peptide. This peptide is based on tetramerization domain of p53, and has the advantage of inhibitory mechanism, which is highly specific and transient.

From the previous studies and my results, the p53 function should need to be not

only up-regulated but also down-regulated. It is important for the concentration of tetrameric p53 to be regulated adequately. This insight about stability and regulation of oligomeric structure should be useful as universal knowledge of other oligomeric proteins.