Internship proposal Identification of novel p53 target by proteomics and transcriptome analyses.

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Abstract: The tumor suppressor gene *p53* is mutated in more than half of all human cancers. Recent cancer genomic analyses have identified a number of genes mutated in cancer tissues, however the mutation of the *p53* gene is still the most common alteration observed in the majority of human cancers. Because over 90% of missense mutations are clustered within its DNA-binding domain, the crucial function of p53 in tumor suppression is considered as a sequence-specific transcription factor. In response to various types of cellular stress, activated p53 regulates many target genes that induce cell cycle arrest, apoptosis, DNA repair, and cellular senescence. We have previously isolated a number of p53 target genes, including p53RDL1, XEDAR, and PADI4 by cDNA microarray analysis. To identify novel p53-target, we conducted two approaches.

a) We performed proteome analysis using HCT116 $p53^{+/+}$ and HCT116 $p53^{-/-}$ cells treated with 2ug/ml of adriamycin (ADR). Cell lysates collected at 0 to 72 hours after ADR treatment were subjected with LC-MS/MS analysis. Among 19,069 peptides identified by this analysis, we screened peptides which were induced by p53 as follows; 1) more than 5 fold higher expression in HCT116 $p53^{+/+}$ cells than those in HCT116 $p53^{-/-}$ cells at 72 hours after ADR treatment. 2) more than 5 fold higher expression in HCT116 $p53^{+/+}$ cells at 72 hours after ADR treatment compared with those at 0 hours. As a result, 131 peptides of 41 proteins including p53 and WAF1 were induced by ADR in a p53 dependent manner.

b) We also conducted whole body transcriptome screening of $p53^{-/-}$ and $p53^{+/+}$ mice with or without X-ray irradiation. Analysis of 240 samples from 20 tissues revealed novel p53-target genes and non-coding RNAs those are induced by p53 in a tissue specific manner. Characterization of one novel p53-target will be the project of visiting student. The aim of this project is functional analysis of novel target in human carcinogenesis. Within a couple of months, visiting student would learn several molecular biological techniques such as, Quantitative Real time PCR, Western blotting, cell culture, reporter assay, and DNA sequencing.

Recent publications from our laboratory:

C. Tanikawa, et al. Nat Genet 44 (2012) 430-434, S431-432.

C. Tanikawa, et al. Nature communications 3 (2012) 676.

V. Kumar et al. Nat Genet 43 (2011) 455-458.

Y. Kamatani, et al. Nat Genet 42 (2010) 210-215.

Y. Kamatani, et al. Nat Genet 41 (2009) 591-595.

C. Tanikawa, et al. Cancer Res 69 (2009) 8761-8769.