〔課程-２〕

審査の結果の要旨

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To better understand the function of p53 in carcinogenesis, this study tried to isolate the novel downstream targets of p53 by differential expression profiles. Elegant experiments were performed to prove the late cornified envelope group I (LCE1) as new p53 target with function of importance.

First, the microarray analysis suggested that the expression levels of *LCE1B* and *LCE1C*, two members of LCE1 family, were induced over seven folds by the exogenous introduction of wild-type p53 in U373MG cells (p53 mutated). By quantitative RT-PCR (qPCR), further investigation showed that besides *LCE1B, LCE1C,*other *LCE* members such as *LCE1E* and *LCE1F* were also up-regulated by the exogenous p53 in U373MG and H1299 (p53 null) cells. Furthermore, DNA damage such as Adriamycin or UV irradiation strongly induced the expression of *LCE1A, LCE1B, LCE1C, LCE1E* and *LCE1F* in a p53 dependent manner. Meanwhile, siRNA of p53 significantly abolished the induction of these genes by DNA damage. It strongly suggested *LCE1B, LCE1C, LCE1E* and *LCE1F* as p53 target genes.

Then, at least three regions containing p53-binding motifs (p53BS2, p53BS6, and p53BS7) were identified with enhancer activity in wide type p53 dependent manner by reporter assay. Specially, p53BS2 in the 5' flanking region of *LCE1F* may associate directly with p53 by chromatin immunoprecipitation (ChIP) assay. In addition, *LCE1F* expression was found much lower in p53 mutant cells compared with p53 wild-type cells by qPCR. Clearly, at least *LCE1F* is a direct target gene of p53.

To search for the interacting protein(s) of LCE1 proteins, HA-tagged LCE1A-F proteins expressed in HEK293T cells were pulled down by HA. Then immunoprecipitants were excised and analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Protein arginine methyltransferase 5 (PRMT5) was found to be one of the best candidates. Subsequent co-immunoprecipitation assay validated the interaction of ectopic LCE1F with both exogenous and endogenous PRMT5. PRMT5 was further shown to co-localize with LCE1F in both the cytoplasm and the nucleus by immunocytochemical analysis.

PRMT5 is one of the best-characterized PRMTs with symmetric di-methyl arginine (sDMA) activity. An important key marker of the PRMT5 activity is the level of symmetric di-methylated histone 3 arginine 8 (H3R8me2s). Through hypermethylation of histone H3R8, PRMT5 could directly silence the tumor suppressor genes such as Retinoblastoma (RB) family proteins by transcription.

Gain of function and loss of function experiments were performed to figure out the role of interaction between LCE1F and PRMT5. The suppression of LCE1 or p53 by siRNAs remarkably increased the methylation level of H3R8me2s specific to PMRT5, while the ectopic LCE1F dramatically decreased the methylation level of H3R8me2s. If this is true, LCE1 will be of great interests as the negative regulator of PRMT5, which is considered as onco-protein with overexpression in various types of human cancer.

In conclusion, here the authors have demonstrated that LCE1 family members of *LCE1A, LCE1B, LCE1C, LCE1E* and *LCE1F* are novel p53 downstream genes, and that at least *LCE1F*, one of the members, is a direct target gene transactivated by p53. Interestingly, LCE1 was found to negatively regulate histone H3 methylation through the interaction with PRMT5.

LCE1 genes are expressed mainly in the outermost layer of the epidermis, suggesting the essential role in maintenance of barrier function. Physiological function of LCE proteins remains largely unknown. Definitely, this study provides us new function of LCE1 as p53 targets, especially its involvement in human cancer for the first time. Future investigations for the will pave the road to reveal the detailed mechanisms of p53 in carcinogenesis. Thus, it deserves to the PhD degree from the University of Tokyo.