Project number 49

Identifying interactors of CHD7 in DNA repair

［1］**Research group**
Principal Investigator (PI)：
Haico. van Attikum
(Leiden University Medical Centere)
Host researcher at IDAC：
Akira Yasui
(IDAC Tohoku University)

Expenditure report of research funds：
Consumables 30,000 YEN

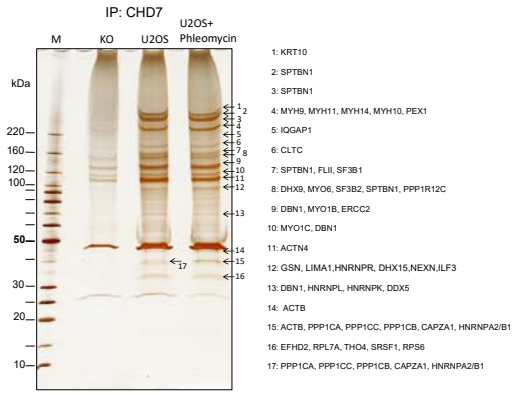
［2］**Research setup**

CHD7 mutations cause CHARGE syndrome characterized by congenital anomalies such as malformations of the craniofacial structures, peripheral nervous system, ears, eyes and heart. Recently we identified CHD7 as a novel factor with an potential role in DNA repair. Discovering new CHD7 interactors may help us to unravel its potential role in DNA repair and disease etiology . In order to determine the proteome around CHD7, Dr. Akira Yasui will support the important step of proteome analysis in our international collaboration.

［3］**Research outcomes**

（3－1）**Results**

Mass spectrometry analysis will be performed after immunoprecipitation of endogenous CHD7 from wild type cells in untreated and phleomycin treated cells to discover new DNA damage dependent interactors of CHD7. CHD7 knock-out cells were prepared as a negative control. This proteomic approach will be performed by the IDAC member group, while verification of interacting proteins in living cells using co-immunoprecipitation experiments will be performed by our group. Two antibodies were compared and one of the two was selected for immunoprecipitation using cell extracts from untreated cells or cells treated with Phleomycin.



The above results of gel electrophoresis of IPed proteins suggest,

1) Most of the IP proteins obtained with the extracts from U2OS cells may be proteins interacting with CHD7 as these were not found when using CHD7 KO cells
2) The proteins immunoprecipitated with CHD7 antibody may be related to the authentic function of CHD7, meaning they may be related to the CHARGE syndrome. Some of the binding proteins are very unique and may be specific for CHD7.
3) Treatment of U2OS cells with Phleomycin did not give rise to unique proteins immuno-precipitated with anti-CHD7, as far as we can detect in the gel electrophoresis. However, most DNA damage influences protein-protein interaction only locally near DNA damages site and therefore only a small fraction of these interactions may be affected. Thus, these data do not inform much on DNA damage-induced CHD7 interactions.

（3－2）**Future perspectives**

In order to determine possible changes in the proteome of CHD7, we need more sensitive assays like label-free or SILAC-based mass spectrometry. Alternatively, we may use western blotting and prope for specific candidate DNA repair proteins following immunoprecipitation of CHD7.

［4］**List of Papers** None.