Applicable Epigenetic Tools in Cancer Research

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ABSTRACT

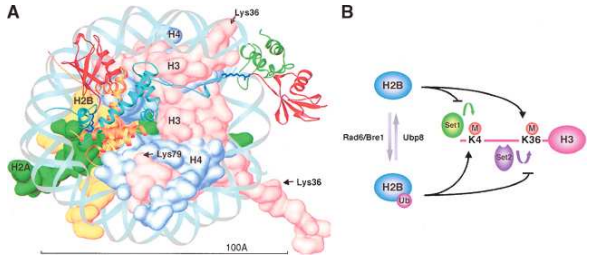
Cancer is a fundamentally genetic disease initiated by alterations/modification in genes, such as oncogenes and tumor suppressors that regulate cell proliferation, survival, and other homeostatic functions. The epigenetic processes, include DNA/nuclear protein methylation, acetylation, phosphorylation, ubiquitylation, sumolyation and RNA interference, are involved in a wide variety of diseases, especially almost all the type of cancers.. Thus, an accurate tool for epigenetic study becomes very important.

Here, we have generated a panel of tools including antibodies and assays for apoptosis phenomena related to epigenetic changes. Human hepatoma cells hepG2 ($1x10^4$/well) were seeded into two of 96-well plate simultaneously. Both plates were treated with 5 µg/ml of camptothecin for 4 hours, The one plate was checked for apoptosis process monitored by the MTT assay, and the other plate was to conduct immuno-fluorescent staining by using a panel of modified histone antibodies, including H3K4[Me1], H3K4[Me2], H3K4[Me3], H3K9[Me3}, ubiquitylation ubH2A, acetylation aceH3K9, phosphorylation phosH3S10. We observed a positive staining on hepG2 by H3K4[Me3], phosH3S10, a weak staining by ubiquitylation ubH2A, H3K9[Me3} and acetylation aceH3K9 which were corresponding to the apoptotic cells, while the higher extents staining (positive) of ubH2A, AceH3K9 on the untreated cells were observed. There was no significant difference in the mono-, di- and tri-methylation of Histone H3K4 among the treated apoptotic cells.

The advances of the epigenetic tools we developed will bring a new perspective insight in the field of apoptosis, signal transduction and cancer research, which will help scientist to open another window to test the epigenetic theory in these fields.

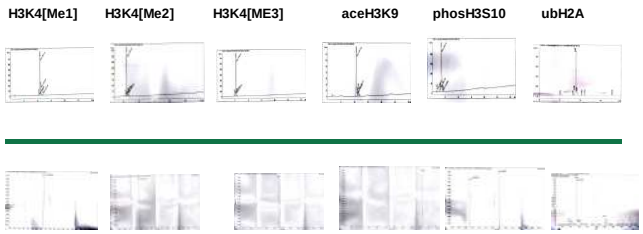
INTRODUCTION

The Histone-Chromatin Structure and Epigenetic Modeling



MATERIALS & METHODS

1: Peptide synthesis and modification



2: Antibody Development and Characterization

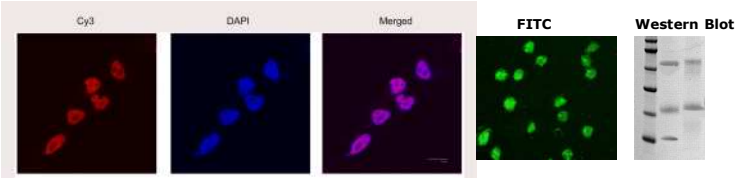
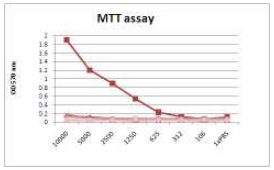


Fig: A representative result: the Camptothecin-treated 3T3 cells was stained by H3K4[Me]3

3:Cell culture of Hep G2 and apoptotic event

Hep G2 cells were grown in DMEM with 4 mM L-glutamine, 1.0 mM Sodium pyruvate, and 10%FBS at 5%CO2, 37oC. When the cells became 80% confluence, lifted cells by Trpsin-EDTA, and seeded into new vessels. All the samples and apoptosis analysis were performed at p4.



MTT assay is available upon request, Please contact us if you have any further questions.

CONCLUSIONS

Cancer is a fundamentally genetic disease initiated by alterations/modification in genes. To monitor the link with the apoptotic events and epigenetic markers may provide a profound understanding for the genetic and epigenetic disease causation in cancer.

REFERENCES

1: Manuel Boix-Chornet, et al. Release of Hypoacetylated and Trimethylated Histone H4 Is an Epigenetic Marker of Early Apoptosis. J. Biol. Chem., Vol. 281, Issue 19, 13540-13547, May 12, 2006

2: AbboMax, Inc Catalogue-Tools for Epigenetic Study, Dec 2008

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