Characterization of the oncogenic functions of Nucleophosmin/NPM1

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学位の種類	博士（　人間生物学　）
学位記番号	博甲第　8311　号
学位授与年月	平成　29 年　3 月　24 日
学位授与の要件	学位規則　第４条第１項該当（昭和２８年４月１日文部省令第９号）
審査組織	グローバル教育院
学位論文題目	Characterization of the oncogenic functions of Nucleophosmin/NPM1 (Nucleophosmin/NPM1 のがん化機能の解明)

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論文の要旨
Abstract of thesis

The doctoral thesis of Lin Jianhuang consists of two projects. One is characterization of the function of Nucleophosmin/NPM1 in NF-κB-dependent gene transcription and the second is functional characterization and efficient detection of Nucleophosmin/NPM1 oligomers. Abstracts of these two projects are as follows,

1. Project 1:

Characterization of the function of Nucleophosmin/NPM1 in NF-κB-dependent gene transcription

1-1. Background

Nuclear factor-κB (NF-κB) is a transcription factor that plays important roles in tumor initiation, promotion, progression, and metastasis. Precise and sufficient gene regulation by NF-κB requires its specific posttranslational modifications and interactions with cofactors. It is reported that Nucleophsomin/NPM1 (NPM1), a multifunctional phosphoprotein that has been implicated in oncogenesis, regulates the expression of the SOD2 gene through activation of the recruitment of NF-κB to SOD2 promoter. However, whether NPM1 regulates NF-κB pathway and if so, what is the mechanism underlying the regulation is still unknown.

1-2. Purpose

In this project, Lin aimed to characterize the function of NPM1 in NF-κB-dependent gene transcription

1-3. Materials and methods

1) The interaction between NPM1 and NF-κB is investigated by immunoprecipitation in HeLa cells, GST pull-down using purified proteins, and proximity ligation assay (PLA) in HeLa cells.
2) The effect of NPM1 on NF-κB-dependent transcription is examined by reporter assay in HeLa cells and MEFs, and by RT-qPCR in HeLa cells and mouse peritoneal macrophages.
3) The genome-wide NF-κB target genes regulated by NPM1 are examined by DNA microarray.
4) The effect of NPM1 on the recruitment of NF-κB to target gene promoters is examined by chromatin immunoprecipitation (ChIP) in HeLa cells.
5) The effect of NPM1 on DNA binding activity of NF-κB is investigated by EMSA.
6) The correlation of NPM1 expression and activation of NF-κB is examined in mouse colon adenomas, and breast cancer cell lines.

1-4. Results

1) NPM1 directly interacts with NF-κB p65 and p50.
2) NPM1 regulates a subset of NF-κB target genes with functional enrichment in inflammation and immunity.
3) NPM1 is important for recruitment of NF-κB to target gene promoters by enhancing the DNA binding activity of NF-κB, however, NPM1 itself releases from NF-κB after NF-κB binds to DNA.
4) NPM1 expression correlates with activation of NF-κB both in colon adenomas and breast cancer cell lines.

1-5. Discussion

In this project, Lin has revealed the role of NPM1 in the NF-κB signaling pathway and the physiological significance of this role. The data suggest a working model in which NPM1 interacts with NF-κB in the nucleus to stimulate the binding of NF-κB to the target gene promoters. Interestingly, NPM1 itself disassociates from NF-κB once NF-κB binds to DNA. NPM1 interacts with the N-terminal DNA binding domain of p65 and competes with DNA for the binding to p65, which results in the release of NPM1 after p65 binds to DNA. These results suggest that the chaperone-like function of NPM1 is working for the maximal transcriptional activity of NF-κB. Considering that NPM1 also shows other oncogenic functions independent of NF-κB, such as regulating the activity and stability of p53 and ARF, cell growth, proliferation, and anti-apoptosis, it is likely that the expression level of NPM1 is a suitable diagnostic marker to determine the aggressiveness of cancer cells and also a suitable target of cancer therapy.

2. Project 2:

Functional characterization and efficient detection of Nucleophosmin/NPM1 oligomers

2-1. Background

NPM1 is a multifunctional phosphoprotein that has been implicated in oncogenesis. Although the effect of oligomer formation on the biochemical activities of NPM1 is not well understood, plenty of observations have suggested that changes of the oligomer formation of NPM1 could influence its biological functions, especially its oncogenic functions. This highlights the importance of monitoring oligomeric state of NPM1 in the cells under different conditions or stimuli for better understanding of the biological functions of NPM1 oligomerization.

2-2. Purpose

In this study, Lin aimed to examine the biochemical function of NPM1 oligomerization in vitro and establish a detection system for cellular NPM1 oligomerization status.

2-3. Materials and methods

1) Examine the binding of WT-NPM1 and monomeric NPM1 mutants to histone by GST-pull-down.
2) Examine nucleosome assembly activity of WT-NPM1 and monomeric NPM1 mutants by EMSA and nucleosome assemble assay using purified core histones and NPM1.
3) Investigate the RNA binding activities of WT-NPM1 and monomeric NPM1 mutants by filter binding and sucrose density gradient assay.
4) For systematic study of the oligomerization of NPM1, Lin utilized the split synthetic Renilla luciferase protein fragment-assisted complementation (SRL-PFAC) bioluminescence system, and established HeLa cell lines stably expressing N-RL-NPM1 and C-RL-NPM1 or N-RL-NPM1 and C-RL.
5) Examine the changes of oligomeric state of NPM1 in cells upon mitotic synchronization, TNF-α treatment, and serum starvation by reporter assay.

2-3. Results

1) Oligomerization of NPM1 is dispensable for histone binding but essential for the nucleosome assembly activity *in vitro*.
2) The split synthetic Renilla luciferase protein fragment-assisted complementation (SRL-PFAC) assay can be used to study the oligomerization of NPM1.
3) TNF-α treatment and mitotic synchronization increased the oligomerization of NPM1, whereas serum starvation decreased the oligomerization of NPM1.

2-4. Discussion

The biochemical function of the oligomerization has been poorly understood. In this study, Lin demonstrated that the oligomerization of NPM1 is required for its histone chaperone activity. Thus, Lin suggested that the histone chaperone function of NPM1 is regulated by monomer-oligomer conversion. The successful detection of the changes of NPM1 oligomerization under different culture conditions indicating that this assay system can be used for systematical study of NPM1 oligomerization in cells under various conditions to understand the functions of NPM1 oligomerization. Moreover, it can also be a promising method for the screening of NPM1 oligomerization inhibitor for cancer therapy.

The changes of NPM1 oligomerization under different cell culture conditions could be caused by posttranscriptional modifications, interaction with nucleoplasmic proteins, or loss of interaction with nucleolar proteins.

審査の要旨
Abstract of assessment result

【批評 Review】

Lin elucidated that the chaperon-like function of NPM1 is required for the optimal activation of NF-κB function. This is a unique finding. There is no previous report indicating the requirement of chaperon-like molecule for transcription factor activities. Analysis of oligomerization status is also interesting. These unique approaches will contribute to the novel understanding of transcriptional regulation and cancer development.

【最終試験の結果 Result】

The final examination committee conducted a meeting as a final examination on 26th January, 2017. The applicant provided an overview of dissertation, addressed questions and comments raised during Q&A session. All of the committee members reached a final decision that the applicant has passed the final examination.

【結論 Conclusion】

Therefore, the final examination committee approved that the applicant is qualified to be awarded Doctor of Philosophy in Human Biology.