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博士の専攻分野の名称	博士（工学）
学位記番号	第 ２２５７４ 号
学位授与年月日	平成21年１月14日
学位授与の要件	学位規則第４条第１項該当 工学研究科生命先端工学専攻
学位論文名	Functional studies of human nucleolar proteins, fibrillarin and nucleophosmin （ヒト核小体タンパク質、フィブリラリンとヌクレオフォスミンの機能解析）
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論文内容の要旨

General introduction

The nucleolus is the most active and dynamic nuclear sub-domain that forms at the end of mitosis around the tandemly repeated clusters of ribosomal DNA (rDNA) gene. The nucleolus is a subnuclear compartment that locally concentrates the transcription and processing machineries that are responsible for generating ribosome subunits. Nucleolus plays a prominent role in the organization of various components of the nucleus. The primary function of the nucleolus is as the site of ribosome biogenesis in eukaryotic cells. The nucleolus organizes the various components of nucleus and considered as plurifunctional. All the functions of nucleolus are achieved by nucleolar proteins. Fibrillarin (FBL) and nucleophosmin (NPM, also known as B23) are abundant nucleolar proteins, which have roles in ribosomal biogenesis. FBL has also role in pre-rRNA processing and embryonic development. NPM is involved in numerous cellular processes including protein chaperoning and centrosome duplication, mouse embryonic development. Thus, although both FBL and NPM are known as multifunctional nucleolar proteins, their roles in the cell-cycle are still unknown.

Functional analysis of a nucleolar protein, FBL in HeLa Cells

Immunostaining shows that the localization of FBL during the cell-cycle of HeLa cells is dynamic. FBL is localized at the chromosome periphery during mitosis. Functional studies by using a combination of immunofluorescence microscopy and RNAi (RNA interference) technique show that depletion of FBL has no effect on the nucleolar structure. However, FBL-depleted cells show abnormal nuclear morphology. Moreover, depletion of FBL results in the reduction of the cellular growth and modest accumulation of cells with 4*n* DNA content and delays mitotic progression. These data suggest that FBL would play a critical role for the maintenance of nuclear shape and cellular growth.

Functional analysis of a nucleolar protein, NPM on mitotic chromosomes in HeLa Cells

To analyze the function on mitotic chromosomes, NPM is depleted by RNAi. Depletion of NPM leads to defects in cell division followed by an arrest of DNA synthesis due to activation of p53-dependent checkpoint response in HeLa cells. Depletion of NPM causes mitotic arrest due to spindle-checkpoint activation. The mitotic cells accumulated by NPM depletion have aberrant chromosomes (misaligned and non-aligned). The chromosome abnormalities occur due to defects in chromosome congression, proper mitotic spindle and centrosome formation, as well as defects in kinetochore-microtubule attachments. Loss of NPM thus causes severe mitotic defects and delayed mitotic progression. These findings indicate that NPM is essential for mitotic progression and cell proliferation.

Functional analysis of a nucleolar protein, NPM in the formation of nucleolar and nuclear structures in HeLa cells

Immunostaing shows that NPM is dynamically localized throughout the cell-cycle of HeLa cells. Localization of NPM at the chromosome periphery during mitosis is confirmed by using a combination of RNAi and 3-D microscopy. Depletion of NPM causes distortion of nucleolar structure as expected and leads to unexpected dramatic changes in nuclear morphology with multiple micronuclei formation. The defect in nuclear shape of NPM depleted cells, which is clearly observed by live-cell imaging, is due to the distortion of cytoskeletal (α-tubulin and β-actin) structure resulting from the defects in centrosomal microtubule nucleation. In addition, the nuclear defects are due to mitotic defects. These results indicate that NPM is an essential protein not only for the formation of normal nucleolar structure, but also for the maintenance of regular nuclear shape in HeLa cells.

General conclusion

The results currently obtained in this study suggest that FBL is a dynamic nucleolar protein and localized at the chromosome periphery during mitosis. FBL, which has no role in the structural integrity of nucleolus, has important roles in the maintenance of normal nuclear morphology and for the cellular growth. NPM is also dynamic nucleolar protein and localized at the chromosome periphery. NPM may interact with FBL and nucleolin during cell-cycle. NPM is required for chromosome congression, proper mitotic spindle and spindle pole formation, and kinetochore-microtubule attachment. Thus NPM has important role in mitotic progression and cell proliferation. Moreover, NPM is required for the structural integrity of nucleolus. NPM also regulate the normal nuclear structure by maintaining the cytoskeletal structure. As both FBL and NPM have roles in cell proliferation, they might be candidates as targets in development of future cancer therapy.

論文審査の結果の要旨

本論文では、ヒト中期染色体のプロテオーム解析により同定された染色体関連タンパク質のうち２種類、すなわちフィブリラリンおよびヌクレオフォスミンの機能解析の結果について述べている。ここで用いた２種類のタンパク質はいずれも核小体に見られるものであり、その染色体に対する機能は従来知られていないものである。

フィブリラリンについては先ず、その細胞周期を通じての動的な局在の変化を明らかにし、細胞分裂期では染色体周辺部に局在する結果を述べている。しかもRNA干渉法を用いてフィブリラリンを細胞から除いた状態を作り出すと、ヒト細胞は正常な核形態および細胞成長をする事ができないのである。すなわち染色体タンパク質としてフィブリラリンは種々の機能を有するのである。

次にヌクレオフォスミンについて同様にRNA干渉法を用いてそれを細胞から除いた状態を作り出すと、核小体および核構造の異常を引き起こす結果が生じる。そこで、フィブリラリンと同様に、その細胞周期を通じての動的な局在の変化を観察すると、ヌクレオフォスミンも染色体周辺部に局在するのである。かつ、ヌクレオフォスミンを除いた状態での染色体構造に関する詳細な観察の結果、ヌクレオフォスミンは正常な紡錘体形成および動原体と微小管の接着に必要なタンパク質である事が明らかになる。すなわち染色体タンパク質としてのヌクレオフォスミンは種々の機

能を有するのである。

以上のように、本論文は染色体タンパク質の機能解明に重要な新しい知見を加える事に寄与し、さらには医療分野への貢献も期待できる。

よって本論文は博士論文として価値あるものと認める。