

Inhibition of cytokinesis by overexpression of NudCL that is localized to the centrosome and midbody

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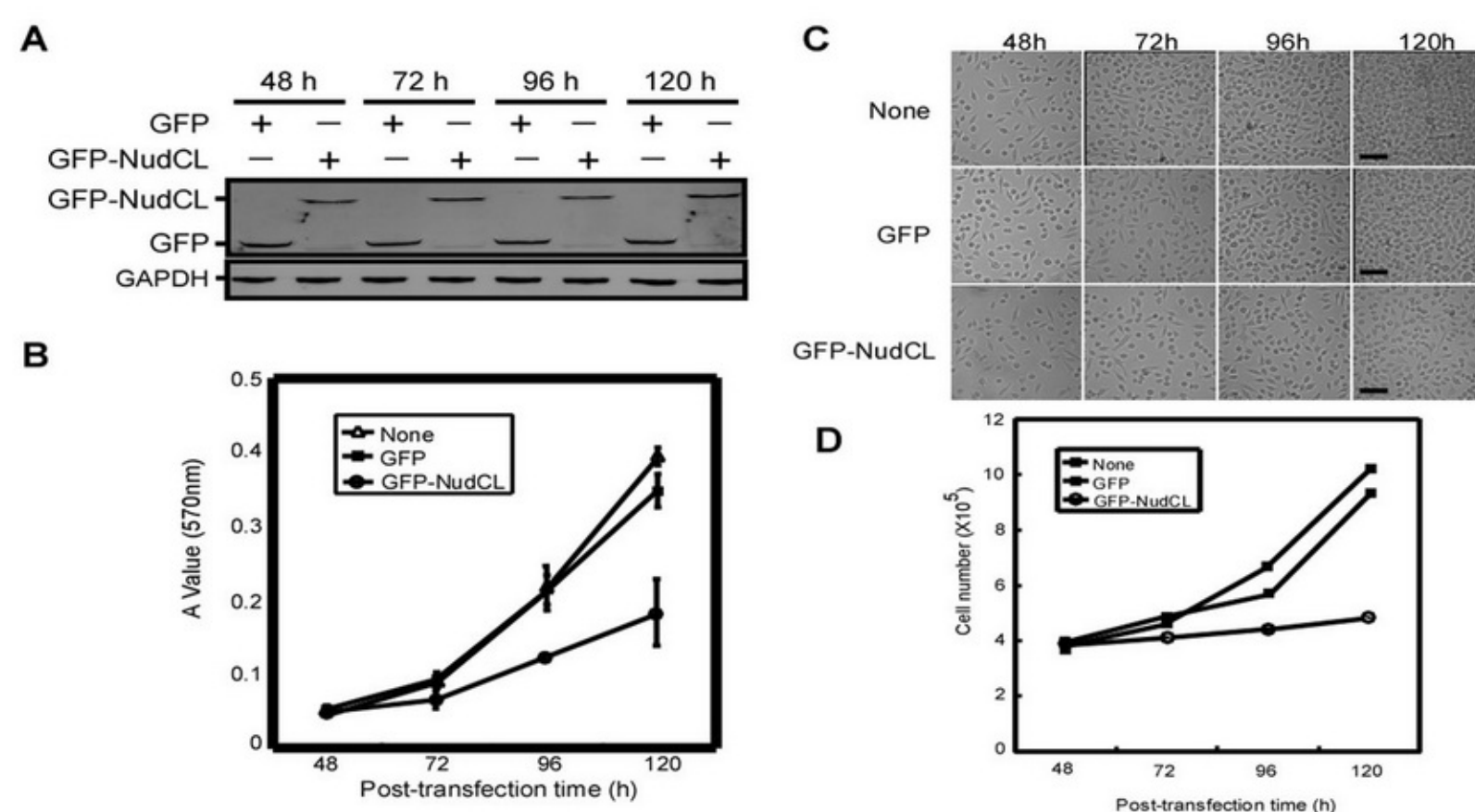
Introduction

Cytokinesis is the process by which a single cell divides into two physically distinct daughter cells; however, the molecular details of this fundamental process remain largely unknown. A large number of proteins are recruited to the midbody during cytokinesis. The midbody concentrates proteins that are associated with vesicular transport, thereby leading to abscission at a site that is adjacent to the midbody. Recently, we and other groups have found that nuclear distribution gene C (NudC) plays an important role in cytokinesis and mitosis in mammalian cells. We further show that depletion of NudCL, a mammalian NudC-like protein, induces multiple mitotic defects. However, little is known about the effects of NudCL overexpression on mammalian cells. Here, we find that overexpression of NudCL that is localized to the centrosome and midbody results in cytokinesis defects and inhibits cell proliferation, suggesting a potential role of NudCL in cytokinesis.

Results

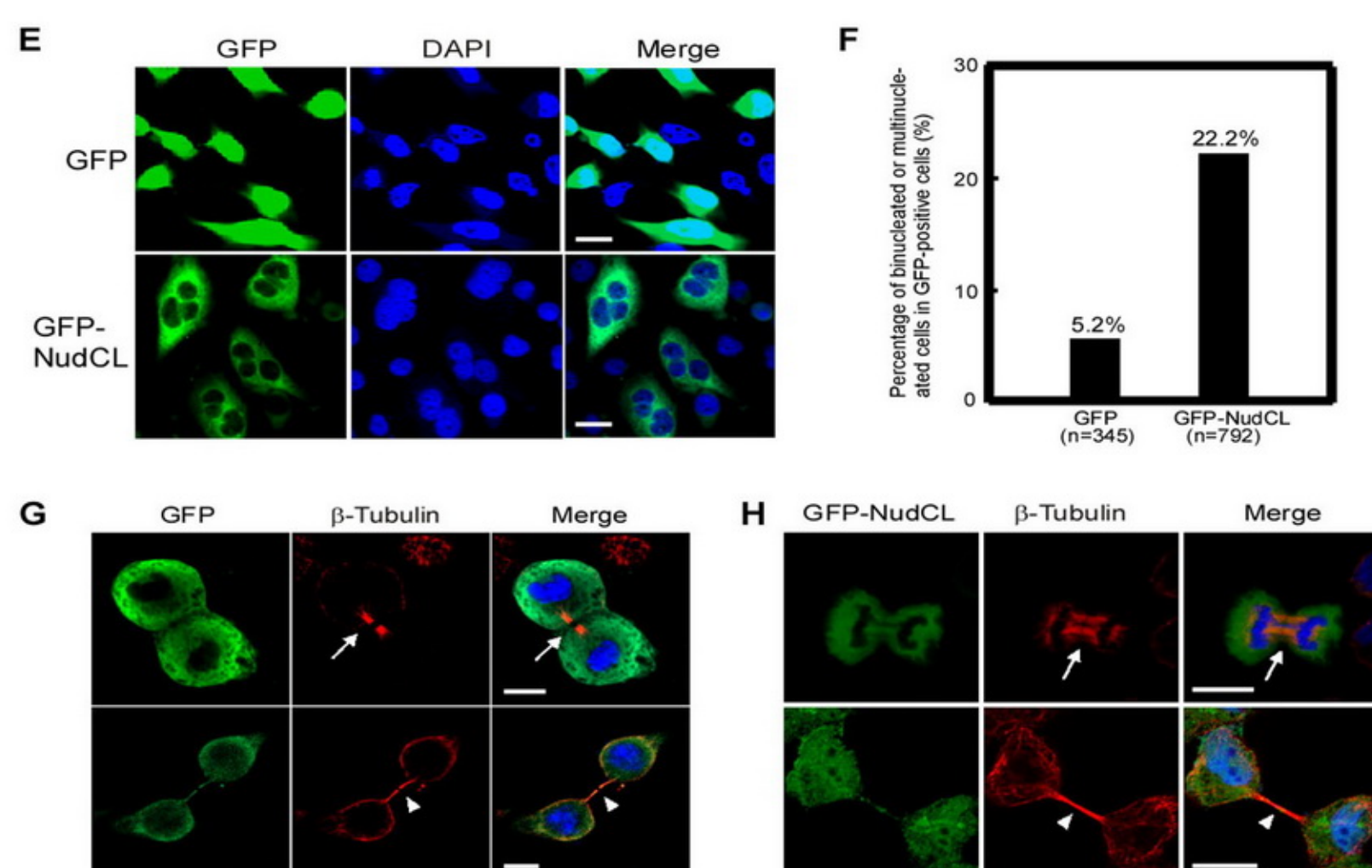
1. Overexpression of NudCL inhibits the cell proliferation

MTT assays showed that overexpression of GFP-fused NudCL (GFP-NudCL) significantly inhibited cell proliferation compared with cells transfected with pGFP vector (Figure A-B), which is supported by the data from cell counting experiments (Figure C-D).



2. Overexpression of NudCL induces multinucleation

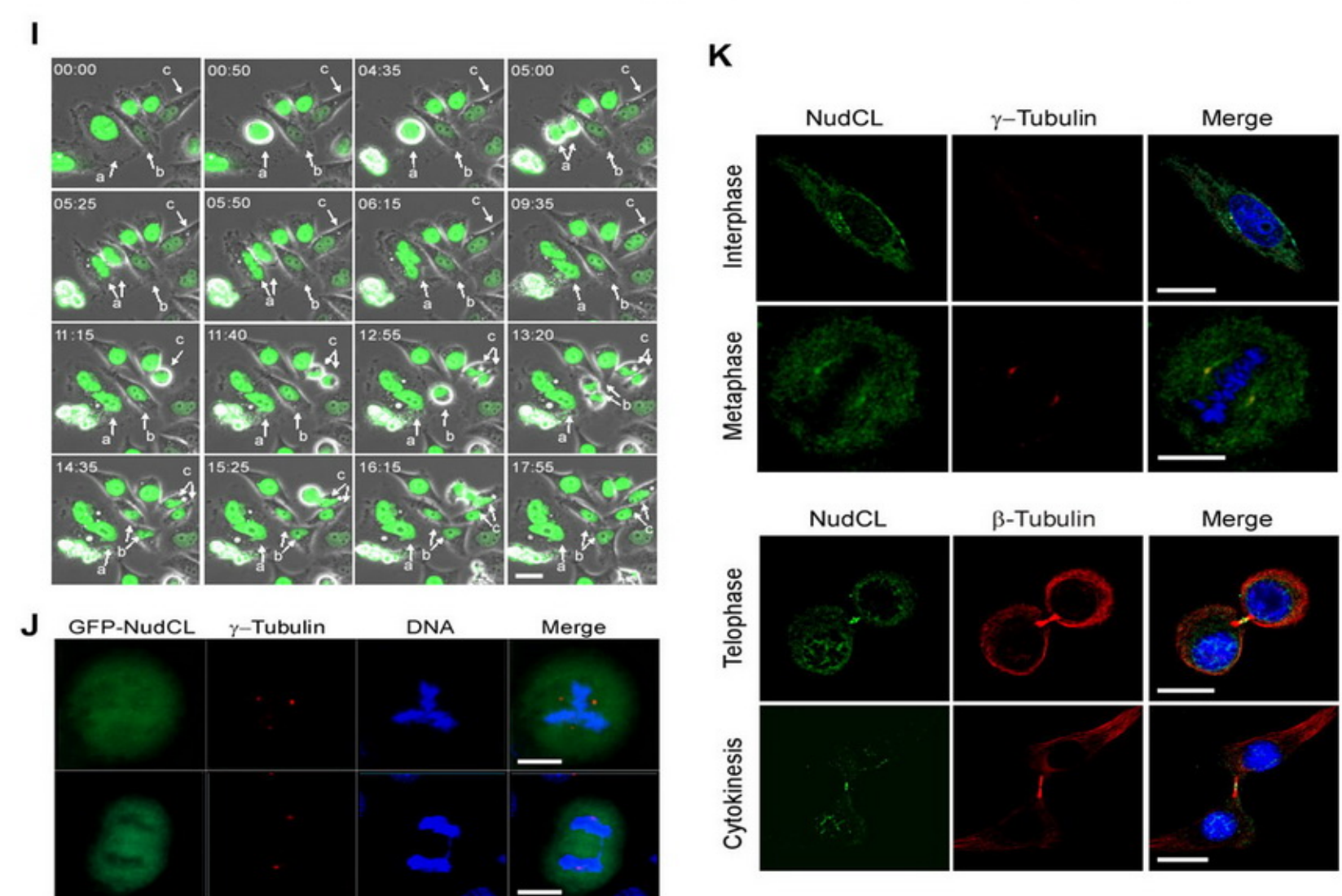
To investigate the mechanism of the inhibition of cell proliferation by NudCL overexpression, we employed immunofluorescence microscopy. The data showed a significant increase in binucleated or multinucleated cells in cells expressing GFP-NudCL (approximately 22%) compared to that in cells expressing GFP (about 5%) (Figure E-F), implying a potential role of NudCL in cytokinesis. Furthermore, overexpression of NudCL resulted in an abnormal structure of microtubule bundles at the cleavage furrow during anaphase and telophase compared to the cells with GFP overexpression (Figure G-H). In the cells with NudCL overexpression, the dark areas corresponding to the midbody at the middle of intercellular bridge in tubulin staining were hardly detectable (Figure H, bottom panel). These data suggest that overexpression of NudCL may inhibit formation of the dense midbody matrix that is essential for the abscission of two daughter cells and consequently the cells fail to complete cytokinesis.



3. NudCL overexpression leads to mitosis and cytokinesis failure

To confirm the cytokinesis defects in cells with NudCL overexpression, time-lapse microscopy was performed to monitor cell cycle progression. The vectors pCFP-NudCL or pCFP were transfected into HeLa cells that stably express GFP-histone H2B. At 48 h after transfection, the cells were imaged for 18 h. In untransfected cells that were indicated by arrows b and c, their daughter cells moved away from each other as soon as the ingress of the cleavage furrow was complete (Figure I). The whole progression of cell division was finished in 3 h. The cells with CFP overexpression exhibited the similar cell cycle progression to the untransfected cells. However, the CFP-NudCL-overexpressing cell indicated by arrow a still remained interconnected after exit from mitosis, and then fused back to become a binucleated cell (Figure I). These data clearly revealed that there are cytokinesis defects in cells with NudCL overexpression.

In addition to cytokinesis defects, overexpression of NudCL also induced multiple mitotic defects. We found that NudCL overexpression resulted in the formation of multipolar spindle and lagging chromosomes (Figure J).



4. NudCL Localizes to the centrosome and the Midbody

To address the reason why overexpression of NudCL induces cytokinesis defects, we examined the subcellular localization of endogenous NudCL. The data showed that NudCL was associated with the centrosome in interphase and spindle poles at metaphase (Figure K, upper panels). During cytokinesis, NudCL was accumulated at the midbody (Figure K, bottom panels). However, in cells with NudCL overexpression, NudCL diffused into the cytoplasm and failed to focus on the centrosome and midbody.

Conclusion

These data show for the first time that NudCL is localized to the centrosome and midbody. Overexpression of NudCL induces multiple defects in mitosis and cytokinesis, and consequently leads to the inhibition of cell proliferation. In cells with NudCL overexpression, NudCL was diffused into cytoplasm and the dense matrix disappeared from the midbody, indicating that NudCL may be important for recruiting the matrix proteins to the midbody. Together with our previous report that depletion of NudCL results in mitotic defects, these data imply that NudCL may play an important role in both mitosis and cytokinesis.

Materials and methods

1. Plasmid Construction

Full-length of human NudCL that had been constructed previously in our laboratory was cloned into the vectors pEGFP-C1 and pECFP-C1 to generate GFP-NudCL and CFP-NudCL vectors, respectively.

2. Immunofluorescence and time-lapse microscopy

HeLa cells grown on coverslips were fixed with cold methanol and stained with anti-NudCL, α or γ -tubulin (Sigma-Aldrich) antibodies for 2 h at room temperature, followed by incubation with either Cy3-conjugated anti-mouse Ig or FITC-conjugated anti-rabbit Ig secondary antibody (Jackson ImmunoResearch) for 40 min. DNA was then stained with DAPI (Sigma). Finally, the mounted coverslips were analyzed by confocal fluorescence microscopy (LSM510, Zeiss). For time-lapse microscopy, HeLa cells transfected with CFP-NudCL for 48 h were incubated in phenol red free DMEM containing 10% fetal calf serum. Cells were then placed in a incubation chamber on Zeiss LSM510 microscope with a motorized stage and observed under reflection or phase with 40X lens. Image sequences were acquired every 25 minutes by cooled CCD camera (Carl Zeiss). Axiovision 4.1 software was used for image acquisition and analysis of live cell data.

3. MTT assays

HeLa cells were transfected with either GFP or GFP-NudCL and pBABE-puro at a ratio of 7 : 1. At 24 h after transfection, puromycin was added to enrich the transfection-positive cells. The cells were reseeded into 96-well plates at 48 h posttransfection and applied for MTT assays at the times as indicated. In brief, MTT solution (5 mg/ml, Bio Basic Inc., USA) was added to each well and cells were incubated for 4 h. Then, dimethylsulfoxide was added into each well. The optical density was determined by Tecan Infinite M200 Multimode microplate reader at 570 nm. Each assay was performed in triplicate and repeated at least three times independently.