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LETTER TO THE EDITOR

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

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Dear Editor,

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 [1]. In animal cells, cytokinesis usually consists of four stages: selection of the cleavage site, assembly of the cleavage furrow, ingression of the cleavage furrow, and abscission of the cell [1, 2]. At anaphase, the spindle midzone microtubules are bundled at their overlapping plus ends and the cleavage furrow has ingressed substantially. In telophase, after the cleavage furrow ingression, the midzone becomes narrow and tightly packed, which forms a dense structure known as the midbody. 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 [3]. During this process, the central spindle microtubules and the proteins localized at the midbody are essential for successful cytokinesis [1].

Recently, we and other groups have found that nuclear distribution gene C (NudC) plays an important role in cytokinesis and mitosis in mammalian cells [4-6]. We further show that depletion of NudCL, a mammalian NudC-like protein, induces multiple mitotic defects [7]. 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.

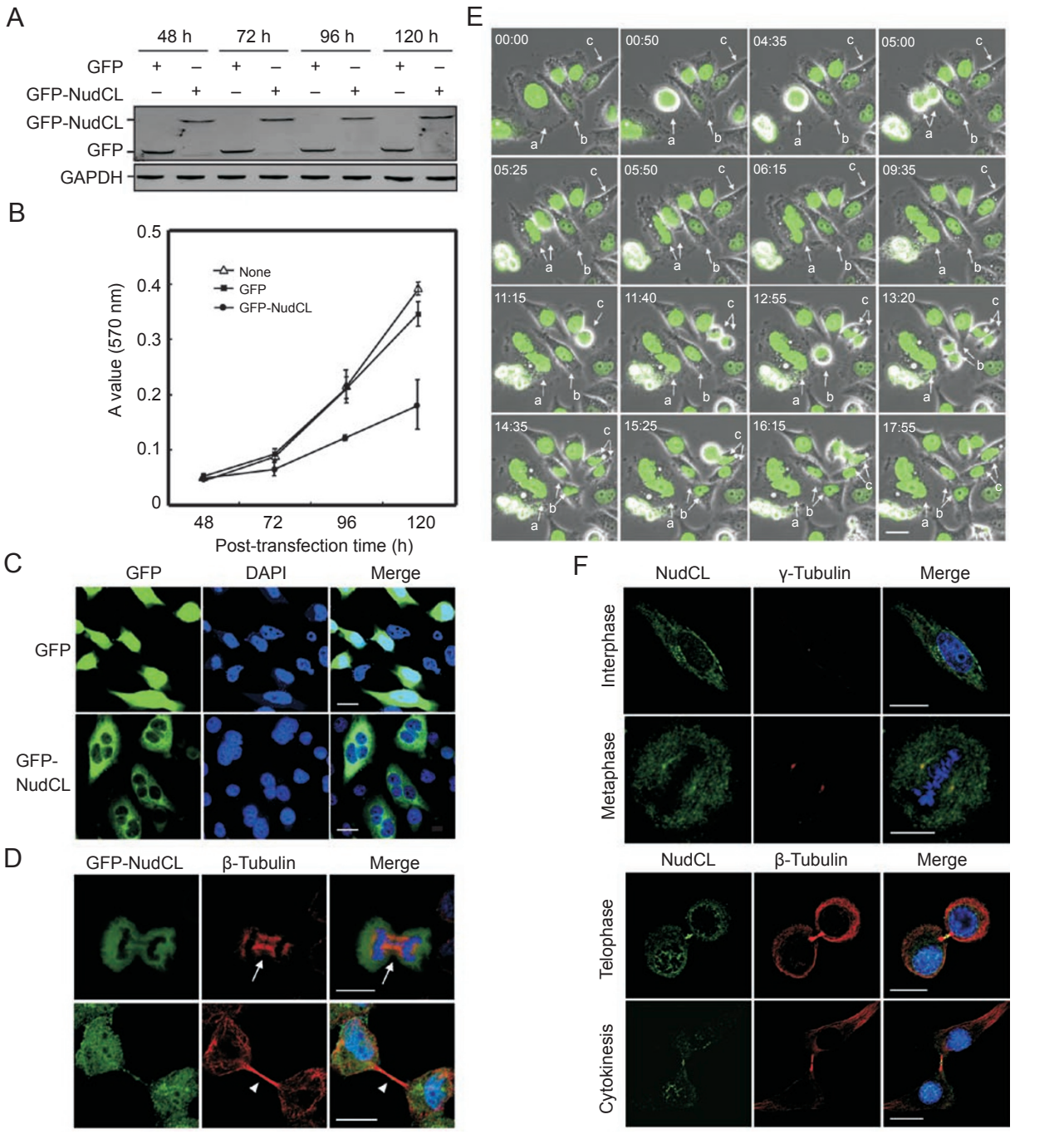
To explore the function of NudCL, we examined the phenotypes of cells with NudCL overexpression. MTT assays showed that overexpression of GFP-fused NudCL (GFP-NudCL) significantly inhibited cell proliferation compared with cells transfected with pGFP vector (Figure 1A-1B), which is supported by the data from cell counting experiments (Supplementary information, Figure S1). Together with our previous data that depletion of NudCL significantly suppresses cell proliferation [7], these findings indicate that a proper level of NudCL is critical for cell proliferation.

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 1C and Supplementary information, Figure S2), 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 1D, upper panel and Supplementary information, Figure S3). 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 1D, bottom panel). The midbody forms at the region of the central spindle microtubules during anaphase and telophase [1]. This structure is associated with the dense matrix, which is obscured by epitope masking in tubulin immunofluorescence studies [8]. 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.

To confirm the cytokinesis defects in cells with NudCL overexpression, time-lapse microscopy was performed to monitor cell cycle progression. The vectors pCFP-NudCL (Cyan fluorescent protein-fused NudCL) or pCFP was transefected 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 ingression of the cleavage furrow was complete (Figure 1E and Supplementary information, Movie S1). 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 (Supplementary information, Movie S2). 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 1E and Supplementary information, Movie S1). These data clearly revealed that there are cytokinesis defects in cells with NudCL overexpression.

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 1F, upper panels). During cytokinesis, NudCL was accumulated at the midbody (Figure 1F, bottom panels). However, in cells with NudCL overexpression, NudCL diffused into the cytoplasm and failed to focus on the centrosome and



midbody (Supplementary information, Figure S4).

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 (Supplementary information, Figure S5). Time-lapse microscopy showed that the CFP-NudCL-overexpressing cell indicated by arrow a entered into M phase at 00:50 and exited from mitosis around 05:25, indicating a mitotic delay (Figure 1E and Supplementary information, Movie S1). These data imply a role of NudCL in mitosis, which is consistent with our previous report [7].

Because of the downregulation of dynein intermediate chain (IC) in cells depleted of NudCL, we tried to determine the protein levels of dynein IC in cells with NudCL overexpression. The result revealed that dynein IC was not significantly changed in the cells transfected with GFP-NudCL compared to that with GFP (Supplementary information, Figure S6).

To our knowledge, these data show for the first time that NudCL is localized to the centrosome and midbody, and plays a potential role in cytokinesis. 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. Consistent with this hypothesis, cells depleted of NudCL failed to form the dense matrix of the midbody and displayed cytokinesis defects, including abnormal elongation of intercellular bridge between two daughter cells (Supplementary information, Figure S7).

The components of the midbody matrix include membrane-trafficking proteins, actin-associated proteins, protein kinases, and microtubule-associated proteins, which are critical for cytokinesis [3]. The emerging data suggest that abscission may be controlled by the centrosomes and a subset of centrosomal proteins [9, 10]. A number of mammalian proteins, such as centriolin, Polo-like kinase 1, Cdc14A, Lats kinase 1 and 2, are shown to localize to the centrosome and midbody and play an important role in cytokinesis, especially in the abscission process [11]. Here, NudCL appears to have similar subcellular localization and function to these proteins. Further studies will be clearly needed to explore the mechanism by which NudCL regulates cytokinesis.

In conclusion, our data show 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. Together with our previous report that depletion of NudCL results in mitotic defects [7], these data imply that NudCL may play an important role in both mitosis and cytokinesis. Experimental materials and methods are described in the Supplementary information, Data S1.

Acknowledgments

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Figure 1 The cellular phenotypes induced by overexpression of NudCL. **(A**-**B)** Overexpression of NudCL inhibits cell proliferation. HeLa cells were transfected with either pGFP or pGFP-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 harvested at the indicated times and subjected to western analysis **(A)**. GAPDH (Glyceraldehyde-3-phosphate dehydrogenase) was used as a loading control. The cells were also reseeded into 96-well plates at 48 h post transfection, and applied for MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assays at the times as indicated **(B)**. The experiments at each time were repeated at least thrice independently. **(C**-**D)** Overexpression of NudCL induces cytokinesis defects. HeLa cells transfected with either pGFP or pGFP-NudCL for 72 h were subjected to immunofluorescence analysis. DNA was stained with DAPI. NudCL overexpression resulted in multinucleated cells **(C)**. The cells with NudCL overexpression exhibited an abnormal structure of microtubule bundles at the cleavage furrow (arrow) and failed to form the dense midbody matrix at the middle of intercellular bridge between two daughter cells (arrowhead) **(D)**. **(E)** Time-lapse video microscopy shows cytokinesis defects in cells with NudCL overexpression. HeLa cells stably expressing GFP-histone H2B was transfected with pCFP-NudCL for 48 h and subjected to time-lapse video recording. One cell transfected with pCFP-NudCL was indicated by a and two untransfected cells were marked b and c, respectively. Images were shown at selected time points. Movies are representative of more than 50 cells analyzed in three independent experiments. **(F)** NudCL is localized to the centrosome in interphase, spindle poles at metaphase and the midbody during cytokinesis. HeLa cells grown on coverslips were subjected to immunofluorence analysis with the indicated antibodies. DNA was visualized by DAPI. Bar, 10 μm.

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(**Supplementary information** is linked to the online version of the paper on the *Cell Research* website.)