Characterization of the kinesin KIF9 in mammalian cell cycle progression

Jordan E. Hoke, Miguel E. Rivera, Alexa M. Billow, Laura Alsina and Nicholas J. Quintyne

Harriet L. Wilkes Honors College
Florida Atlantic University, Jupiter, FL

#2244 L16

Abstract

The kinesin family of microtubule motors is divided into subfamilies based upon structure and function. KIF9 is the founding member of the Kinesin-9 family, a largely uncharacterized group of kinesins. It was originally identified by sequence homology to other kinesins and shown to interact with the Ras-like GTPase Gem (Piddini et al., 2001). Subsequent studies have indicated that KIF9 is vital for flagellar movement in Trypanasoma brucei, likely via interaction with dynein (Demonchy et al., 2009) as well as podosome regulation (Cornfine et al., 2011). Additionally, Kinesin-9 family members have also been proposed to function in proper microtubule organizing center (MTOC) positioning and timely mitotic entry. We have examined KIF9 function in mammalian cells by using siRNA-mediated knockdown and overexpression. Our analysis has focused on normal progression through S phase and subsequent transition to, and progression through, mitosis. We have seen several effects on cell cycle progression. First, a transfected, synchronized population of cells shows a notable delay in mitotic entry. Second, there is a delay in normal S phase progression. Third, we see that there is a change in mitotic index and decrease in the number of anaphase and telophase cells. Finally, we see an increase in the rate of multinuclearity, a hallmark of failure of cytokinesis. Taken together, we propose that KIF9 is required for normal entry and completion of mitosis, possibly via regulation of the contractile ring.

Introduction

The kinesin superfamily of microtubule motors is divided into subfamilies based upon structure and function. Kinesins share an evolutionarily conserved motor domain (Nakagawa et al., 1997), which can vary in position along the polypeptide. Searches of the human and mouse genomes identified several kinesins by sequence homology (Nakagawa et al., 1997) leading to organization of the proteins into 14 families (Miki et al., 2001). KIF9 is the founding member of the Kinesin-9 family, a largely uncharacterized group of kinesins. It was originally shown to interact with the Ras-like GTPase Gem (Piddini et al., 2001), with the implication that it was required to bridge and coordinate the microtubule and actin cytoskeleton, a vital interaction for normal mitotic progression. Subsequent studies have indicated that KIF9 is vital for flagellar movement in *Trypanasoma brucei*, likely via interaction with dynein (Demonchy et al., 2009) as well as podosome regulation (Cornfine et al., 2011). Additionally, Kinesin-9 family members have been proposed to function in proper microtubule organizing center (MTOC) positioning and timely mitotic entry in *Dictyostelium discoideum* (Tikhonenko et al., 2009).

Methods

•*Cell Culture.* COS-7 cells were grown in Dulbecco's Modified Eagle Medium supplemented with 10% FBS and Penicillin-Streptomycin. Cells were grown at 37°C in 5% CO_2. The cells were passaged with 0.05% Trypsin-EDTA.

•*Antibodies.* The following primary antibodies were used: mouse anti-PCNA (Source, Dilution), mouse anti-Nek2 (source, dilution), mouse anti-α-tubulin (DM1A; source, dilution) , rabbit anti-γ-tubulin (S/D). Secondary antibodies were Texas-Red-X or Alexa488 conjugated goat anti-mouse or anti-rabbit (S/D).

•*Plasmids.* Combinations of shRNA plasmids from the set NM_022342 were used to knock down KIF9 expression. The anti-sense KIF9 siRNA sequences are

5'-CCGGCGCCAGTACCTTAAAGGACAACTCGAGTTGTCCTTTAAGGTACTGGCGTTTTTG-3'
and
5'-CCGGCCCAGTTAGAAGAAACGCTATCTCGAGATAGCGTTTCTTCTAACTGGGTTTTTG-3'

The plasmid vector pLKO.1-puro confers resistance to puromycin, allowing for stable selection for the presence of shRNA plasmid after transfection.

•*Transfection.* For each coverslip 3 µl of FuGene 6 was added to 100 µl of Opti-MEM. After incubating for 5 minutes, 3 µL of of each shRNA-plasmid was added and after 15 minutes, the mixture was added dropwise to the cells on the coverslip. After 24 hours, media with 2 mg/ml puromycin was added to select for cells that retained the plasmid. The puromycin-containing media was refreshed after 24 hours and replaced with normal medium for an additional 24 hours prior to fixation.

•*Immunofluorescence.* Fixed cells were stained with antibodies as described in Quintyne et al. 1999. Cells were incubated with either DAPI or Hoescht in order to stain chromosomes. An Olympus IX-81 Inverted Fluorescence Microscope was used to analyze the immunofluorescent cells. Between 200 and 300 cells were analyzed per slide and the experiments were performed twice.

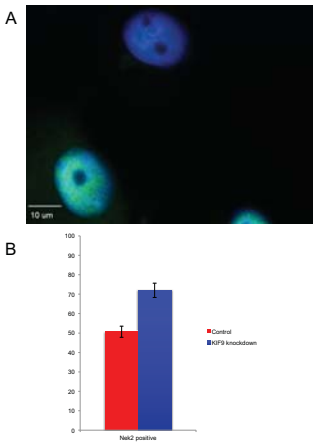

Figure 1: Percentage of S phase cells in control and KIF9 knockdown cells. (A) Cells were scored for PCNA nuclear enrichment. PCNA: green, DAPI: blue. Bar = 10 µm.
(B) Percentages of cells positive for PCNA nuclear staining was scored for both conditions. Error bars indicate SD.

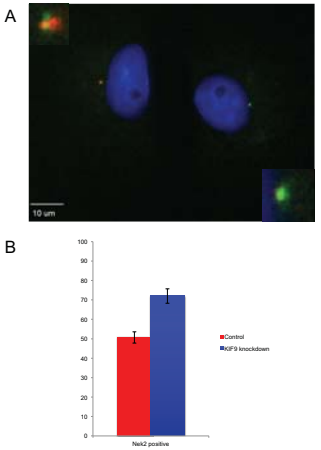

Figure 2: Percentage of S phase cells in control and KIF9 knockdown cells. (A) Cells were scored for Nek2 centrosomal localization. Nek2: green, γ-tubulin: red, DAPI: blue. Insets are 3X magnifications. Bar = 10 µm.
(B) Percentages of cells positive for Nek2 centrosomal localization was scored for both conditions.

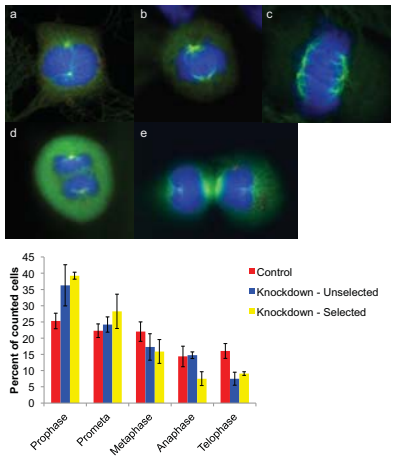

Figure 3: Mitotic progression. (A) Mitotic cells were scored for each stage: (a) prophase, (b) prometaphase, (c) metaphase, (d) anaphase and (e) telophase in control and knockdown cells. MTs: green, γ-tubulin: red, DAPI: blue. (B) Percentages of mitotic population in each stage were calculated for control and siRNA-treated cells.

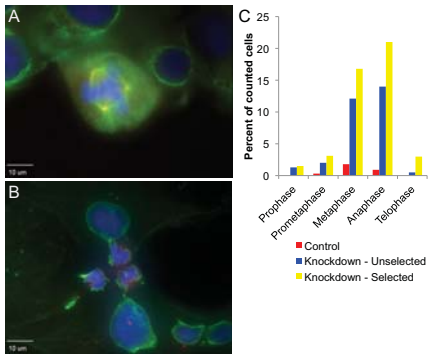

Figure 4: Multipolar cells were observed in the population, with many seen in (A) metaphase and (B) anaphase. MTs: green, γ-tubulin: red, DAPI: blue. Bar = 10 µm.
(B) Percentages of mitotic population for each stage that exhibited multipolarity in control and siRNA-treated cells.

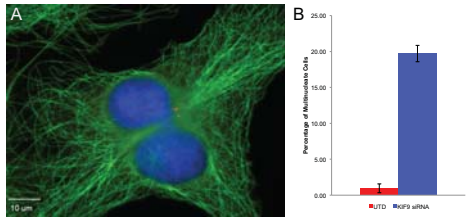

Figure 5: Multinuclearity. (A) representative multinucleate cell seen in KIF9 knockdown. MTs: green, γ-tubulin: red, DAPI: blue. Bar = 10 µm. (B) Percentage of cells population for each stage that exhibited multipolarity in control and siRNA-treated cells.

Conclusion

- KIF9 knockdown has an effect on normal cell cycle progression into mitosis. This is like other members of the kinesin-9 family members previously reported. Knockdown cells show a statistically significant increase in both PCNA- and Nek2-positive cells ($p<0.02$ and $p<0.0001$ respectively).
- Knockdown of KIF9 leads to an increase in the prevalence of cells in the early stages of mitosis, with fewer cells found later in mitosis.
- KIF9 knockdown leads to a large increase in multipolarity
- An increase in rates of multinuclearity is also seen when KIF9 is knocked down, suggesting early mitotic exit.

- Taken together, our data suggest that KIF9 is required for normal mitotic entry and progression and in its absence, cells have greater difficulty entering mitosis and are likely to exit from mitosis prior to completion. This may be due to KIF9 interacting with the cell cortex (via Gem) or another microtubule-based function during late mitosis.

References

Cornfine, S., et al. (2011). The kinesin KIF9 and reggie/flotillin proteins regulate matrix degradation by macrophage podosomes. *Mol. Biol. Cell* 22: 202-215.

Demonchy, R., et al. (2009). Kinesin 9 family members perform separate functions in the trypanosome flagellum. *J. Cell Biol.* 187: 615-622.

Miki, H., et al. (2001). All kinesin superfamily protein, KIF, genes in mouse and human. *Proc Natl Acad Sci USA* 98: 7004–7011.

Nakagawa, T., et al. (1997). Identification and classification of 16 new kinesin superfamily (KIF) proteins in mouse genome. *Proc Natl Acad Sci USA* 94: 9654–9659.

Piddini, E., et al. (2001). The Ras-like GTPase Gem is involved in cell shape remodeling and interacts with the novel kinesin-like protein KIF9. *EMBO J.* 20: 4076-4087.

Tikhonenko, I., et al.(2009) Microtubule-Nucleus Interactions in *Dictyostelium discoideum* mediate by central motor kinesin. *Eukaryotic Cell*, 8: 723-731.

Acknowledgements

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