Deciphering the dual function of RhoGDI2 in Cancer Biology

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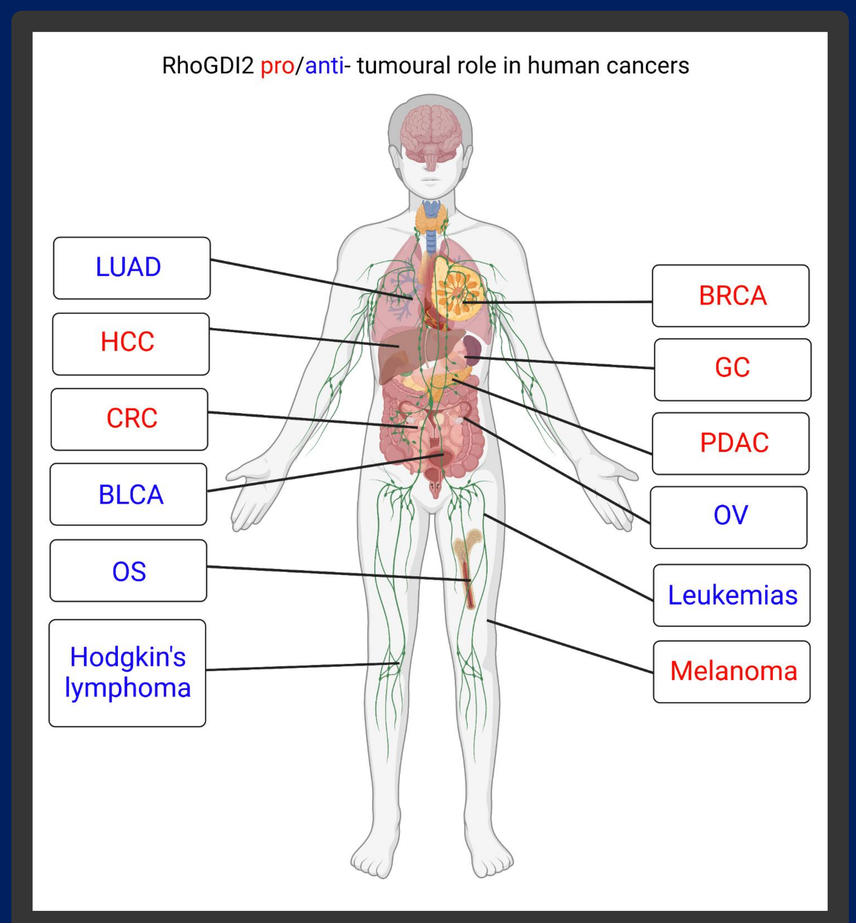
INTRODUCTION

Rho GTPases control a wide variety of signaling pathways to regulate actin and microtubule cytoskeleton thereby defining the cell shape and migration. They also regulate vesicular transport, cell division, and gene transcription. Rho guanine nucleotide dissociation inhibitor 2 (or RhoGDI2) is a regulator of Rho GTPases wherein it not only inhibits them, but also shuttles their inactive forms towards membranes for activation and can also protect them from proteasomal degradation.

RhoGDI2 was initially identified in hematopoietic cells where it localizes in the cytoplasm and was later found to be differentially expressed in other cell types and tissues, including several human cancers where its expression can be correlated to either good or bad prognosis depending on cancer type.



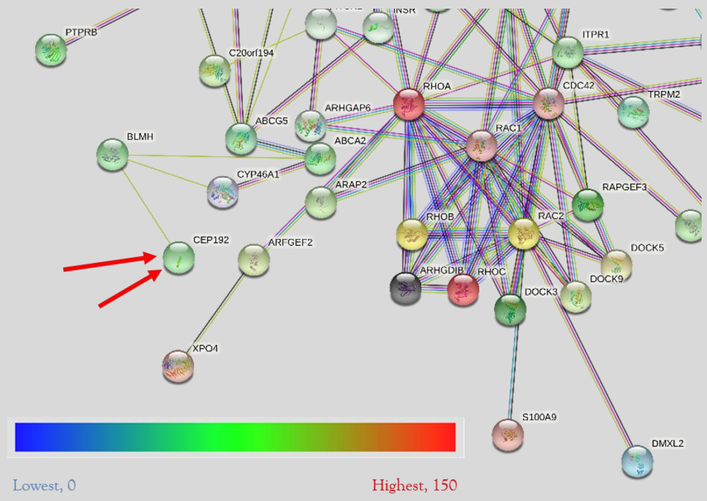
OBJECTIVES

This study explores how RhoGDI2 acts as a double-edged sword in cancer biology by:

- Investigating novel functions of RhoGDI2 in cancer cells
- Identifying the role of RhoGDI2 in immune response

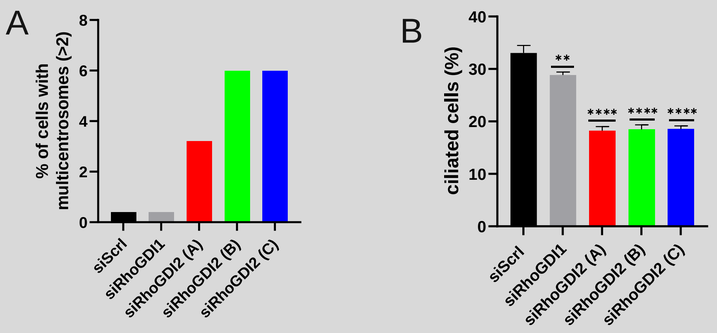
RESULTS

1. Centrosomal proteins were found in our IP/MS aimed at finding RhoGDI2 interactors



We performed an IP/MS (immunoprecipitation/mass spectrometry) to identify protein interactors of RhoGDI2 to look for its novel functions. Majority of candidates were involved in **cytoskeletal organization and cell motility.** Surprisingly, one such interactor that is been investigated is centrosomal protein 192 (CEP192) which is involved in centrosome-primary cilium complex and spindle formation.

2. RhoGDI2 silencing affects the formation of centrosome-primary cilium complex



We observed that RhoGDI2 silencing affected the centrosome-primary cilium complex, an organelle with multiple cell regulatory functions, including reception and transduction of extracellular signals.

A. The supernumerary centrosome phenotype was enhanced in U-2 OS cells arrested at G2/M phase when the expression of RhoGDI2 was reduced while silencing of RhoGDI1 had no effect.

B. Upon reducing the expression of RhoGDI2 in MG-63 cells, we observed that the formation of primary cilia by the cells had decreased by 2 folds. Again, such effect was not observed upon silencing RhoGDI1.

Although the induced phenotype is not identical in the two cell lines, this data clearly illustrates the implication of RhoGDI2 in the regulation of the centrosome-primary cilium complex.

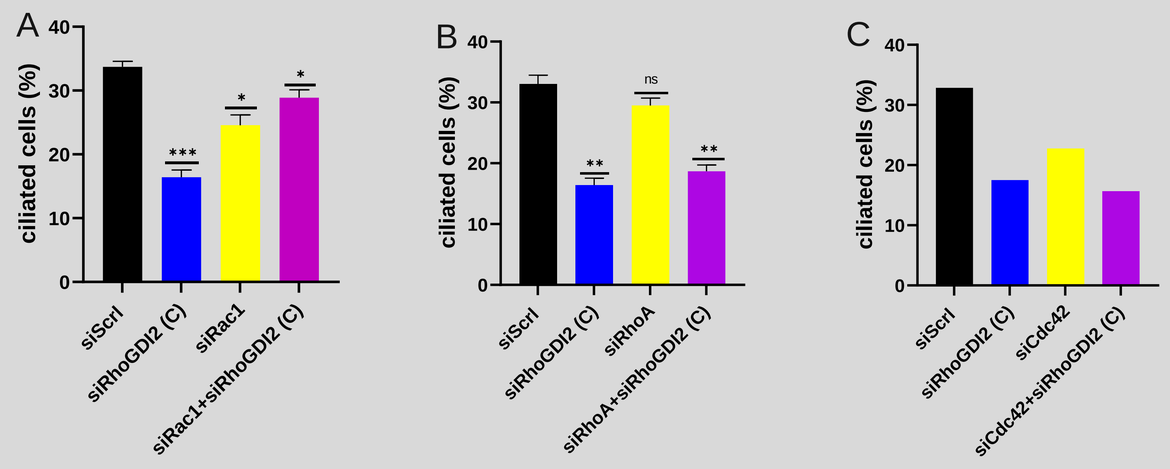
3. Cosilencing of RhoGDI2 with Rac1, but not RhoA, rescues the primary cilia phenotype

As we observed that RhoGDI2 silencing decreases the ability of MG-63 cells to undergo ciliogenesis, we wondered if cosilencing with key Rho GTPases would influence this phenotype.

A. It was rescued that cosilencing of RhoGDI2 and Rac1 in MG-63 cells rescues the ciliogenesis phenotype lost by RhoGDI2 silencing.

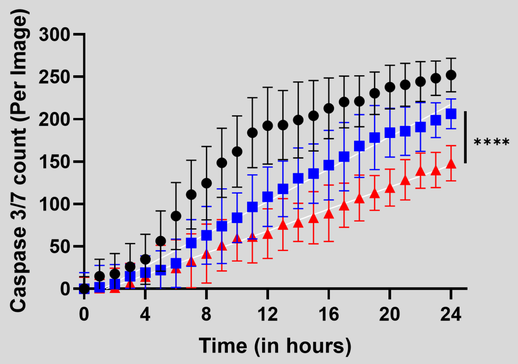
B. Cosilencing of RhoGDI2 with RhoA did not seem to have any affect on the primary cilia phenotype in MG-63 cells.

C. Preliminary results with cosilencing of RhoGDI2 and Cdc42 also do not show any changes in the primary cilia phenotype.



4. Knocking down RhoGDI2 expression in immune cells significantly reduces their tumor killing ability

There are strong similarities between the molecular mechanisms underlying the functions of both the immune synapse and primary cilium. Based on this and on the fact that RhoGDI2 is highly expressed in immune cells, we then verified the implication of RhoGDI2 in the cancer cell killing ability of NK cells.



Co-cultures were established between U2OS cancer cells and NK-92 natural killer cells transfected or not with a control siRNA (siScrl) or with siRhoGDI2. The killing capacity of NK-92 cells was significantly affected upon silencing of RhoGDI2.

OUTLOOK

In conclusion, this study shows that knocking down the expression of RhoGDI2 reduces cancer cell proliferation. Upon looking at potential interactors of RhoGDI2, we observed several proteins involved in cytoskeletal organization and centriole duplication. Moreover, we noticed that RhoGDI2 silencing affected the formation of the centrosome-primary cilium complex, an organelle with multiple cell regulatory functions, including reception and transduction of extracellular signals. Lastly, we also show that suppressing the expression of RhoGDI2 in immune cells negatively influences their ability to target tumor cells. Altogether our data would explain why RhoGDI2 could be considered both as “pro-tumor” by stimulating cancer cell proliferation, but also as “anti-tumor” by participating in cancer cell killing by immune cells.

FUTURE

- Validate the interaction between RhoGDI2 and CEP192.
- Investigate the downstream effects of primary cilia dysregulation due to RhoGDI2 silencing – spindle apparatus formation and autophagy.
- Explore if the influence of RhoGDI2 silencing on immune response depends on Rho GTPases.

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