Deciphering the dual functions of RhoGDI2 in Cancer Biology

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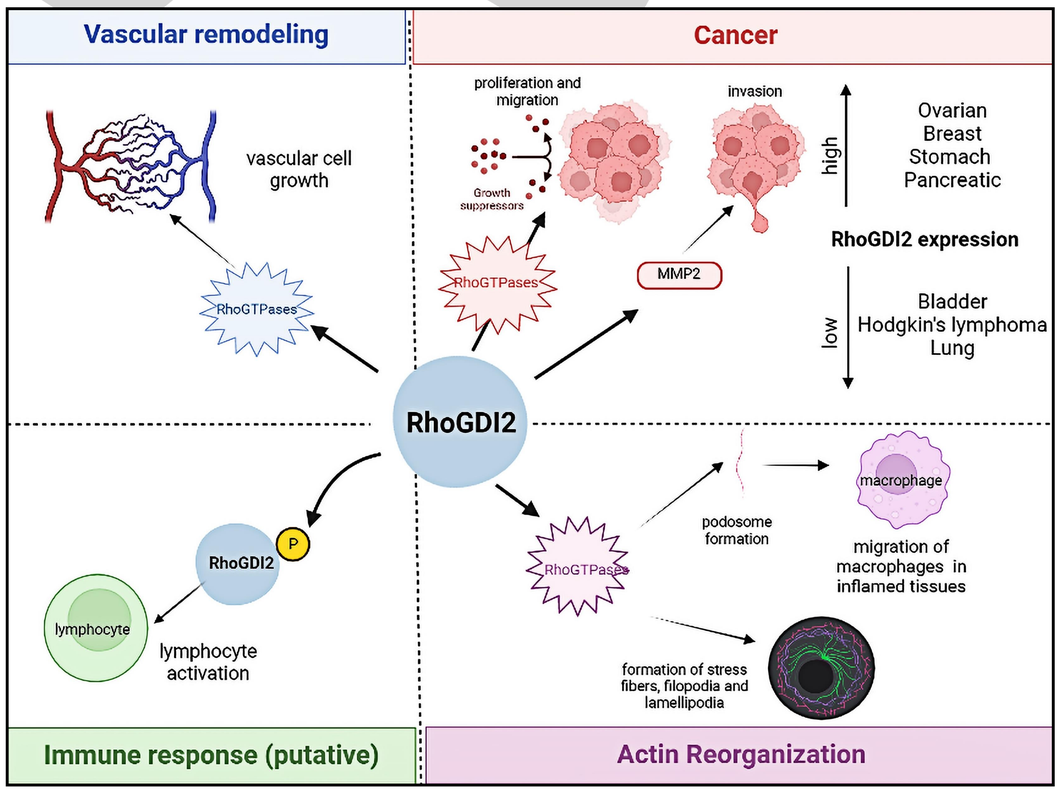
INTRODUCTION

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, neuronal development and transcriptional regulation. RhoGDIs (Rho guanine nucleotide dissociation inhibitors) not only act as inhibitors to Rho GTPases, but they also shuttle inactive Rho GTPases between membranes for their activation and can also protect some Rho GTPases 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 has been correlated to either good or bad prognosis depending on the cancer type and stage.



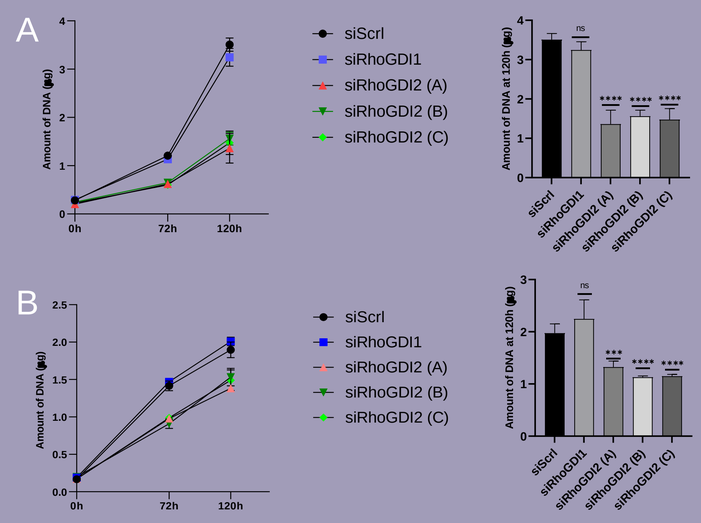
OBJECTIVES

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. Knocking down RhoGDI2 expression in cancer cells decreases their proliferation rate



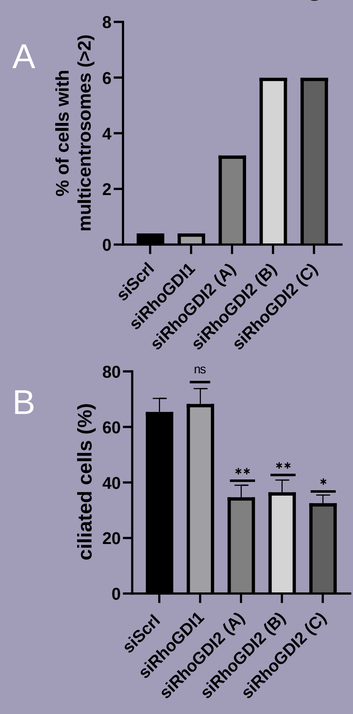
We observed that repressing RhoGDI2 expression by three different siRNA in (A) U-2 OS (osteosarcoma) and (B) PC-3 (prostate adenocarcinoma) human cancer cells significantly reduced their proliferation rate. Such effect was not observed when the closely related RhoGDI1 (73.6% similarity) was silenced, underlining the relevance of our findings.

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

Protein	Uniprot ID	Relevance
Xin actin-binding repeat-containing protein 2	A4UGR9	Cytoskeletal organization
Dynein heavy chain 14, axonemal	Q96DT5	Cytoskeletal organization
Centrosomal protein of 192 kDa	Q8TEP8	Mitotic centrosome and spindle assembly
Microtubule-actin cross-linking factor 1	Q9UPN3	Cytoskeletal organization
Centrosomal protein of 104 kDa	O60308	Ciliogenesis
Actin-related protein 3B	Q9P1U1	Actin cytoskeleton

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**, **cell motility** and, surprisingly, **centriole duplication**.

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



We noticed that centrosomal proteins are potential interactors of RhoGDI2. Following this, 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.

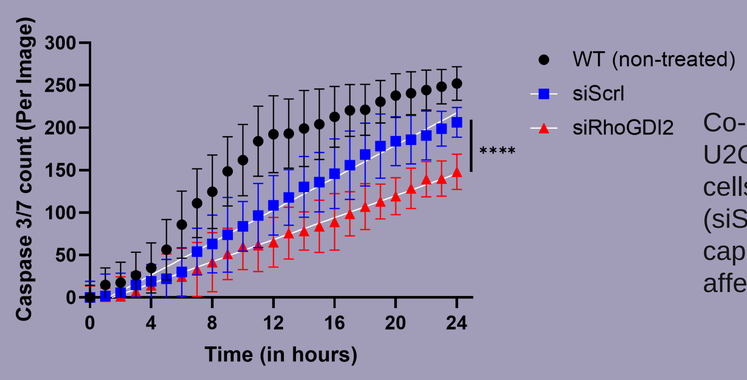
(A) The supernumerary centrosome phenotype was enhanced in U-2 OS cells arrested at G2/M phase when the expression of RhoGDI2 was reduced while silencing of RhoGDI1 had no effect.

(B) Upon reducing the expression of RhoGDI2 in RPE-1 (retinal pigment epithelial cells – which is an excellent model to study this phenotype), 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 models, this data clearly illustrates the implication of RhoGDI2 in the regulation of the centrosome-primary cilium complex.

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 effects of RhoGDI2 silencing on primary cilia formation in cancer cells.
- Investigate the downstream effects of primary cilia dysregulation due to RhoGDI2 silencing – spindle apparatus formation, autophagy and hypoxia.
- Look into the effects of suppressing the expression of RhoGDI2 on the formation of immune synapses.
- Explore if the influence of RhoGDI2 silencing on centrosome-primary cilium complex and immune response depends on Rho GTPases.

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