available at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejconline.com

RhoGDI signaling provides targets for cancer therapy

Michael A. Harding [a,d], *Dan Theodorescu* [a,b,c,*]

[a] *Departments of Urology, University of Virginia, Charlottesville, Virginia, USA*
[b] *Molecular Physiology and Biological Physics, University of Virginia, Charlottesville, Virginia, USA*
[c] *Paul Mellon Urologic Cancer Institute, University of Virginia, Charlottesville, Virginia, USA*

ARTICLE INFO

Article history:
Received 2 February 2010
Accepted 16 February 2010

Keywords:
Guanine Nucleotide Dissociation Inhibitors
RhoGDIs
RhoGDI1
RhoGDI2
RhoGTPase
Cancer therapy
Angiogenesis
Pancreatic cancer
Breast cancer
Bladder cancer

ABSTRACT

Rho GDP-Dissociation Inhibitors (RhoGDIs) are important regulators of the Rho family of small GTPases. The expression of RhoGDIs is altered in a variety of cancers and they have been shown to mediate several processes during tumorigenesis and cancer progression. Using examples of RhoGDI-mediated signaling and expression patterns in endothelial cells as well as pancreatic, breast, and bladder cancer, the multitude of potential cancer therapeutic targets presented by a better understanding of their function is illustrated. Several novel therapeutic strategies are proposed for intervening in RhoGDI signaling, and potential complications arising from their implementation are discussed.

Published by Elsevier Ltd.

1. Introduction

Involvement of the Rho family of small GTP binding proteins in cancer is well established. A great deal of work has been done on the proteins that regulate their GTPase activity and the downstream effectors that are involved in cancer phenotypes. Much of this literature concerns the regulation of Rho activity by the GEFs (Guanine Nucleotide Exchange Factors) and GAPs (GTPase Activating Proteins).[1,2] But a third type of protein, the Rho family GDP Dissociation Inhibitors (RhoGDIs), are just now being appreciated as critical components of the Rho regulating machinery, and an emerging collection of evidence suggests that their activity is altered in carcinogenesis and tumor progression. As more evidence of their multi-faceted influence on the cancer phenotype via Rho family members accumulates, many intriguing and promising therapeutic targets are emerging from the elucidation of how these proteins work. However, an examination of what we now know about RhoGDIs also indicates that their functions may result in tissue-specific effects and thus more extensive understanding of the pathways they regulate will be necessary for the complete realization of novel cancer treatments based on their function.

* *Corresponding author:* Address: Department of Molecular Physiology and Biological Physics, P.O. Box 800422, University of Virginia, Charlottesville, VA, 22908 USA. Tel.: +1 434 924 0042; fax: +1 434 982 3652.
E-mail addresses: mah2n@virginia.edu (M.A. Harding), dt9d@virginia.edu (D. Theodorescu).
[d] Tel.: +1 434 982 3347; fax: +1 434 243 6937.
0959-8049/$ - see front matter Published by Elsevier Ltd.
doi:10.1016/j.ejca.2010.02.025

2. The RhoGDI family and their activities

Although RhoGDI proteins have been reviewed extensively elsewhere,[3–5] a brief summary is necessary for understanding the potential opportunities and pitfalls for RhoGDI-centric intervention. Three RhoGDI proteins have been indentified in humans. They are referred to in the literature by various nomenclatures, but here they will be called RhoGDI1, 2, and 3. The prototype of the family, RhoGDI1, is known as RhoGDIα, arhgdi alpha or arhgdia. RhoGDI2 is variously called RhoGDIβ, arhgdi beta, arhgdib, ly-gdi, and D4-gdi. RhoGDI3 is also known as RhoGDIγ, arhgdi gamma or arhgdig. RhoGDI3 is the most divergent of the three and is associated with the golgi and vesicular membranes.[6] Very little is known about RhoGDI3 in cancer; therefore, it will not be discussed further here. RhoGDI1 is the most ubiquitously expressed of the family. While the highest expression of RhoGDI2 is in cells of hematopoietic origin, it has recently been found in a variety of other tissues and cancers.[3]

RhoGDIs regulate a multitude of phenotypes including cell division, morphology, migration, vesicular trafficking and gene expression. They probably affect these diverse phenotypes principally by controlling the location and activity of members of the Rho family of small GTPases. Therefore, a full appreciation of the function of RhoGDIs is dependent on knowledge of the role of RhoGTPases, which have been reviewed extensively elsewhere.[1,2,7,8] Rho proteins comprise a family within the larger superfamily of Ras-related proteins. They are signal transducers that alternate between an activated, GTP-bound state, and the inactive, GDP-bound form. The cycle between the GTP or GDP bound state is mediated by GTPase activator proteins (GAPs) and guanine nucleotide exchange factors (GEFs), which facilitate the exchange of GDP for GTP. When bound to GTP, active Rho proteins bind to variety of downstream effector molecules. Currently, the Rho family is thought to contain 20 members,[8] and over 60 Rho effectors have been identified.[1] This accounts for the number of signaling pathways regulated by RhoGTPases, and the complexity of the potential impact of RhoGDIs on signal transduction.

RhoGDIs are thought to affect Rho proteins in several ways. In general, RhoGDI and RhoGDI2 have been thought of as multi-modal inhibitors of the RhoGTPases. Methods of Rho inhibition by RhoGDIs include inhibition of Rho catalytic activity by inhibiting the GTPase function as well as inhibiting dissociation of GDP from the Rho protein after hydrolysis. RhoGDIs also interact with prenylated Rho proteins in a manner that removes them from membranes, causing a re-localization to the cytoplasm. In a related function, they are thought to shuttle Rho proteins between membrane domains. Therefore, they cannot be viewed simply as inhibitors of RhoGTPases, but probably also direct activated Rho proteins to certain sub-cellular compartments.[9,10] By virtue of binding Rhos, they are also believed to restrict interactions of RhoGEFs and RhoGAPs with Rho proteins, and the binding of other modulators of Rho activity.

Many of the common Rho family of proteins have been shown or are suspected to interact with one or more of the RhoGDIs (Table 1 in[3]), and it seems likely that additional members of the Rho family will be found to be regulated by GDIs. Differences in the RhoGDIs are reflected in differing affinities for Rho family members. For example, RhoGDI2 has a much lower affinity for Cdc42 than for the Rac proteins, whereas RhoGDI1 has similar affinities for RhoA, Rac, and Cdc42.[11,12] In addition to their innately different affinities for RhoGTPases, RhoGDI1 and RhoGDI2 also exhibit quite different effects on Rho family protein activation in some circumstances, which will be discussed further below.

3. Regulation of RhoGDI effector interactions

Post-translational modifications such as phosphorylation of RhoGDIs are thought to regulate their interactions with their target effectors. Binding and displacement of RhoGDIs from Rho proteins are coordinated with phosphorylation events to carry out complex cellular signaling. An example of these complex signaling cascades in angiogenesis was recently reported by Elfenbein et al.[13] Polarized Rac1 activation in endothelial cells causes migration and is necessary for angiogenesis, and Rac1 activation is preceded by activation of RhoG. FGF2 is a potent endothelial cell motility factor that is a ligand for syndecan 4 (S4). It was shown that RhoGDI1 binds in a complex with S4 and its adaptor synectin, and that this binding increases the affinity of RhoGDI1 for RhoG. Upon ligand binding, and S4 clustering, protein kinase Cα (PKCα) is activated which phosphorylates RhoGDI1 at Ser^{96}. Phosphorylation of RhoGDI1 inhibits its binding to RhoG, resulting in activation of RhoG and the subsequent activation of Rac1. Surprisingly, phosphorylation of RhoGDI1 at Ser^{96} did not change the affinity of RhoGDI1 for Rac1. Knezevic et al.[14] also found that phospho-Ser^{96} did not alter the activation of Rac1 in endothelial cells. However, the same group has shown that RhoGDI1 is phosphorylated at Ser^{96} by PKCα in response to thrombin engagement of endothelial cell surface Protease-Activated Receptor 1 (PAR-1). This phosphorylation results in the release of RhoA by RhoGDI1 and activation of RhoA, which is required for thrombin-induced vascular permeability.[14,15] In these examples, RhoGDI1 directly and indirectly regulates three different RhoGTPases, in two signaling pathways, in one cell type.

Such involvement of RhoGDI1 in angiogenesis and vascular permeability, two hallmarks of the neo-vasculature induced by tumors, illustrates the target-rich therapeutic environment created by a better understanding of RhoGDI function. The ligand/receptor interactions, PKCα, and the RhoGDI1/Rho protein interactions all become viable candidates for inhibiting tumor-induced angiogenesis. This understanding could allow rational and selective drug development. For example, PKC inhibitors could be screened for ability to inhibit the Ser^{96} phosphorylation on RhoGDI1, while minimizing inhibition of PKC activity on other substrates. Hence, knowledge of RhoGDI signaling could lead to more effective, better tolerated angiogenesis inhibitors.

Other kinases, including P-21 Activated Kinase 1 (PAK1) and Src, phosphorylate RhoGDIs are likely to regulate RhoGDI signaling pathways as complex as those regulated by PKCα. PAK1 phosphorylates RhoGDI1 at two sites, which causes the selective release of Rac1, but not RhoA.[16] Interestingly,