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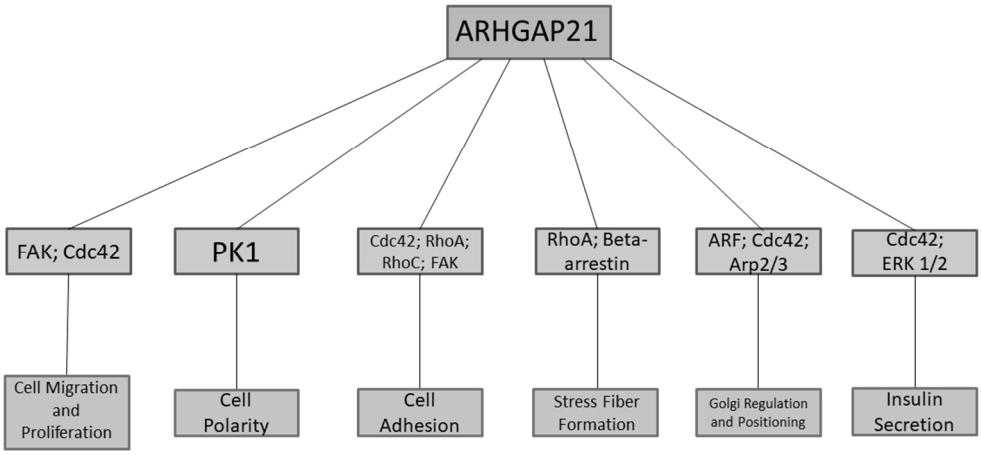

ARHGAP21 and multiple roles it is implied in and the interactors through which it acts.

ARHGAP21 as a master regulator of multiple cellular processes

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Abstract

The cellular cytoskeleton is involved with multiple biological processes and it is tightly regulated by multiple proteins and effectors. Among these, the RhoGTPases family is one of the most important players, which are, in turn, regulated by many other elements. On the past decade, one of those regulators has been overlooked, despite being implied to have an important on many of those processes: ARHGAP21. In this paper, we aimed to review the literature available regarding ARHGAP21, in order to highlight its importance and the mechanisms of action that have been found so far for this still unknown protein involved with cell adhesion, migration, golgi regulation, cell trafficking and even insulin secretion.

Introduction

Ras is a superfamily of proteins in which the RhoGTPases family has a central role due to its importance in cytoskeleton-related processes as demonstrated by their interactions with Rho, Rac and Cdc42 (Hall, 2012). They act like molecular switches, cycling between a GDP-bound (Inactive) form and a GTP-bound (active) form. When GTP-bound, Rho GTPases interact with effectors, triggering cellular processes. The first and most important result of Rho GTPase signaling is the reorganization of the cellular cytoskeleton, mostly through their interaction with mDia and ROCK (Rho-associated kinase), as well as activation of Arp2/3 (Hall, 2012). The cytoskeleton, in turn, is an important element in multiple cellular processes, including migration, proliferation, adhesion, endocytosis and exocytosis.

Due to their importance, Rho GTPase activity is strictly regulated through three factors: guanine nucleotide exchange factors (Rho-GEFs), guanosine nucleotide dissociation inhibitors (Rho-GDIs) and Rho-GTPAse activating proteins (Rho-GAPs) (Hodge and Ridley, 2016). GEFs lead to the exchange of GDP to GTP, acting as positive controllers that stimulate Rho GTPase action. GDIs act as negative controllers by sequestering the GDP-bound form of Rho GTPases, impairing GEFs's ability to activate them. Finally, GAPs also act as inhibitors of Rho-GTPase by promoting the catalysis of GTP into GDP, inactivating the Rho-GTPase after their activation by GEFs and promoting the cycling for correct Rho-GTPase function.

Among the multiple existing Rho-GAPs, this review will focus on ARHGAP21, an overlooked and understudied member of the family with documented relevancy in multiple biological processes ranging from cellular migration to insulin secretion. ARHGAP21 (also known as ARHGAP10) was identified by Bassères D.S. et al in 2002 (Basseres et al., 2002) and has a Rho-GAP domain, a pleckstrin homology (PH) domain, and a PDZ domain. Ever since, it has been the subject of only a few studies, despite being associated with multiple cellular processes (Dubois et al., 2005; Ferreira et al., 2015; Lazarini et al., 2013; Menetrey et al., 2007; Wang et al., 2012).

ARHGAP as a regulator of cytoskeletal processes in cancer cells

ARHGAP21's main interactors are Cdc42 (Dubois et al., 2005), RhoA (Lazarini et al., 2013) and ARFs, especially ARF1 and ARF6 (Klein et al., 2006; Menetrey et al., 2007) all of which are involved with cytoskeleton rearrangement and/or intracellular transport. These are the main proteins which ARHGAP21 interacts with and modulates in the multiple processes it is implied.

A significant amount of the literature available refers to the role of ARHGAP21 in many cancer-related subjects like migration and adhesion, indicating that the protein might be a tumoral suppressor. ARHGAP21 has been identified as a cancer-related gene (Katoh and Katoh, 2004) and is over-expressed in cancer cells (Carles et al., 2006). ARHGAP was foind to interact with Focal-Adhesion Kinase (FAK), with its depletion increasing cell migration (Bigarella et al., 2009). The loss of ARHGAP21-FAK interaction led to increased FAK and Cdc42 activation and, thus, the increase in migration (Bigarella et al., 2009). Thus, ARHGAP21 was identified as a potential tumor suppressor, being able to control the progression of the disease. ARHGAP21 depletion also increased cell migration, as well as decreased proliferation, in PC3 adenocarcinoma cell lines through its activity as a Rho-GAP (Lazarini et al., 2013).

A recently discovered signaling pathway, named “Lateral Signaling”, was also found to be related with migration, with ARHGAP21 playing a key role (Zhang et al., 2016; Zhang and Wrana, 2016). This lateral signaling pathway controls cell polarity and morphology which, in turn, affect migration. This pathway consists of Prickle 1 (Pk1), an important cell polarity protein, and ARHGAP21. ARHGAP21 interacts with Pk1, regulating RhoA (Zhang and Wrana, 2016). This mechanism is important to control the volatility of cell shape, which in turn for regulates cell motility (Zhang and Wrana, 2016).

Another important process on the development of cancer is cell adhesion – or, rather, the lack of (Okegawa et al., 2004), and ARHGAP21 is also important for normal cell-cell adhesion. ARHGAP21 moves from the nucleus to the cellular junctions during cell adhesion, reducing Cdc42 activity (Barcellos et al., 2013). Knockdown of ARHGAP21 reduced the strength of cell-cell adhesion and, in turn, increased migration (Barcellos et al., 2013). ARHGAP21 is a necessary protein for α-tubulin acetylation, possibly by aiding the organization and stabilization of tubulin, and pointed the lack of alfa-tubulin acetylation as a contributing factor for the alterations of migration behavior in cells lacking ARHGAP21 (Barcellos et al., 2013). The study, however, did not find an expected increase in epithelial-mesenchymal transition (EMT) in ARHGAP21-lacking cells; instead, the ARHGAP21-less cells displayed reduced EMT. This is due to a possible role of α-tubulin acetylation in EMT. ARHGAP21 is, therefore, important for both adhesion and migration, and has an interesting relationship with α-tubulin (Barcellos et al., 2013). This is supported by other studies, in which LYL1 knockdown mice led to a reduction of ARHGAP21 and, consequentially, increased vascular permeability in the lungs of mice (Pirot et al., 2014). Therefore, ARHGAP21 is implicated in multiple cytoskeleton-related processes, like adhesion, proliferation and migration, through its interaction with Cdc42, RhoA, RhoC, and FAK.

Finally, ARHGAP21 is also involved in the formation of stress fibers (Anthony et al., 2011; Pirot et al., 2014). In stress fiber formation by RhoA-mediated angiotensin stimulation, ARHGAP21 modulation of RhoA can be controlled by its interaction with beta-arrestin (Anthony et al., 2011). When the beta-Arrestin/ARHGAP21 complex was disrupted, ARHGAP21 activity increased, leading to RhoA inhibition and less stress fiber formation and actin reorganization (Anthony et al., 2011).

ARHGAP21, cellular transport and Golgi

ARHGAP21 is also involved with the regulation of the Golgi apparatus and with the transport of substances – endogenous or exogenous – within the cell. ARHGAP21 was identified as a link between ARF and the downstream activation of Cdc42 in the regulation of Golgi function, which then leads to the modulation of Arp2/3 and F-actin (Dubois et al., 2005). It has been suspected for a long time that ARF coordinated Cdc42 and RhoA for the regulation of Golgi, but the connection between them had eluded researchers until this work came out (D'Souza-Schorey and Chavrier, 2006; Stamnes, 2002). The team was capable to demonstrate that ARHGAP21 interacts with both ARF and Cdc42, establishing the link. A subsequent study pointed that ARHGAP21 physically interacts with ARF through its PH domain and an adjacent alfa-helix (Menetrey et al., 2007).

ARHGAP21 depletion reduced Golgi's ability to be positioned correctly (Hehnly et al., 2010). This positioning process is dependent on dynein and on microtubule, and is controlled by Cdc42 which is, in turn, modulated by ARHGAP21, ARF1, and a vesicle-coating protein coatomer.

On top of its direct action over Golgi-related processes, ARHGAP21 is also involved in the traffic of substances within the cell. The retrograde transport of *E. Coli*'s Shiga toxin through the secretory pathway is modulated by Rho GTPase activity, especially Cdc42 (Hehnly et al., 2009). ARHGAP21 knockdown (or the constitutive activation of Cdc42) inhibited the transport of Shiga toxin, and the addition of the toxin by itself lowered the levels of Cdc42 due to the increase in ARHGAP21 – thus, ARHGAP21 seems to be important for the transport of the toxin within the cell, through its modulation of Cdc42 (Hehnly et al., 2009). The transport of Influenza Virus Neuraminidase was also modulated by ARHGAP21 (Wang et al., 2012). The intracellular transport of influenza virus neuraminidase is crucial for the virus' life cycle, and the depletion of ARHGAP21 (or the constitutive activation of Cdc42) stimulated neuraminidase transport to the cell membrane. Silencing ARHGAP21 led to the increase of viral replication (Wang et al., 2012). Thus, ARHGAP21 modulation over Cdc42 signaling appears to be important in influenza virus infection through its influence over neuraminidase transport.

ARHGAP21 and insulin secretion

Insulin secretion, specifically its second phase, in which secretion is less intensive but long-lasting, is a process that depends on actin rearrangement (Kalwat and Thurmond, 2013). The cortical actin acts as a barrier that prevents the passage of insulin granules coming from the cytoplasm to the membrane and, thus, this cytoskeletal barrier needs to be rearranged. Glucose stimulation of the pancreatic beta cell promotes that rearrangement through multiple pathways, one of the most important involving Cdc42 (Nevins and Thurmond, 2003).

Considering the involvement of the actin cytoskeleton in insulin secretion and the importance of Rho GTPases in the modulation of actin (and the already established role of Cdc42 in insulin secretion), it was not a stretch to imagine that ARHGAP21 could play a role in insulin secretion. Thus, our group decided to investigate that possible involvement. We found is that ARHGAP21 is present in the beta cell (Ferreira et al., 2015), and ARHGAP21 knockdown increased basal insulin secretion, but not glucose-stimulated insulin secretion, as expected. ARHGAP21 knockdown also led to an increase in ERK1/2 phosphorylation, which was previously implied in actin rearrangement during insulin secretion (Kalwat and Thurmond, 2013; Longuet et al.,

2005). These findings suggest, therefore, that ARHGAP21 seems to be involved in insulin secretion. However, its role as well as the underlying molecular mechanisms involved in this process remaining still unclear.

Concluding remarks

As we have shown in this review, ARHGAP21 appears to be an important protein in multiple processes, especially those linked with the actin cytoskeleton including migration, adhesion, intracellular transport and insulin secretion. Through ARHGAP21's modulation of Rho GTPases, especially RhoA and Cdc42, and its interaction with other proteins, like ERK and FAK, ARHGAP21 positions itself as a key member in the regulation of those processes. Despite its importance, ARHGAP21 has been a neglected protein since its discovery, and holds an incredible potential yet to be clarified by future studies.

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