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CDC42 Regulatory Patterns Related To Inflammatory Bowel Disease and Hyperglycemia

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Abstract

As a member of the rat sarcoma virus homolog (Rho) guanosine triphosphatases (GTPases) family, Cdc42 represents a "switch" molecule, by changing from inactive (GDP-associated) to active form (GTP-associated) and vice versa. Cdc42 is activated by the guanine nucleotide exchange factors (GEFs), in contrast to GTPase-activating proteins (GAPs) which are responsable for formation of GDP-binding, inactive form of Cdc42. Some of the fundamental cellular functions are regulated by Cdc42 such as cytosceleton dynamics, cell cycling, transcription and cellular trafficking. In the gastrointestinal system, Cdc42 participates in maintenance of the functional epithelial barrier by controling intestinal epithelial cell polarity and interconnections. In addition, Cdc42 expression in pancreatic β-cells is of great importance for glucose-stimulated insulin secretion. From the pathophysiological point of view, literature data provide some evidence for Cdc42 sigaling in inflammatory bowel disease, as well as in hyperglycemic conditions related to diabetes mellitus. However, whether and by which mechanism Cdc42 contributes to the IBD patophysiology in hyperglycemic conditions is still not fully understood. Therefore, we performed bioinformatics analysis to predict transcriptional factor-gene interactions related to Cdc42 signaling in inflammatory bowel disease in hyperglycemic conditions. In silico analysis predicts various interactions between input genes and output transcriptional factors, and therefore reveals the molecules with the highest predicted effect on particular genes. Based on the predictive interactions with the intracellular molecules, carefully designed *in vitro* or *in vivo* studies are required to get better insight in the pathways of interest. Better understanding of Cdc42 molecular pathway in inflammatory bowel disease and hyperglycemia will help identifying potential targets for therapeutical modifications in clinical setting resulting in better control of the disease progression.

Keywords: Cdc42; Diabetes; GTPase; GTPase-activating protein; Guanine nucleotide exchange factor; Guanosine triphosphatases; Hyperglycemia; Inflammatory bowel disease; Network analysis; Small GTPase; Transcription factors.

Introduction

Cell division cycle 42 (Cdc42) molecule belongs to the rat sarcoma virus homolog (Rho) guanosine triphosphatases (GTPases) family, also known as small GTPases [1]. Rho GTPases act as "molecular switches" by transforming from inactive (associated with GDP) to active state (associated with GTP) aimed to regulate different cellular functions. Cdc42 is involved in fundamental processes for cell survival including cell migration, endo-

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and exocytosis, transcription, and cell cycle regulation [2]. In particular, actomyosin cytoskeleton dynamics, regulated by Cdc42, are of special importance for cellular function, especially in terms of epithelial turnover and integrity. In addition, by modulation of actin remodeling, Cdc42 regulates the proper polarity of the cells, including the distinction between apical and basolateral membranes. Formation of filopodia and stem cell proliferation are also among the important processes regulated by Cdc42 [3]. It has been found that one of the predominant locations of Cdc42 in the cell is the Golgi complex [4]. Furthermore, an active form of Cdc42 has been shown to associate with the COPI complex (Coat Protein Complex I) and therefore mediate retrograde transport from the Golgi apparatus to the endoplasmic reticulum (ER) [5]. Accordingly, it may be stated that Cdc42 participates in cellular trafficking. The explanation for this phenomenon lies in the association between Cdc42 and COPI since COPI represents a protein complex located in Golgi-origin transport vesicles and mediates Golgi apparatus-ER retrograde transport [6]. Taken together, it can be said that the Cdc42 molecule participates in both cellular architecture and motility.

Previous literature, mostly based on *in vitro* studies, reported the importance of Cdc42 for various physiological processes, including mammalian gland function and lactation, platelet aggregation, and glomerular filtration [7-9]. In addition, glomerular podocyte function, neuronal morphology, T-cell activation and migration, and antigen presentation by dendritic cells are some of the important processes mediated by Cdc42 [10-13]. From the aspect of pathophysiology, it has been reported that the Cdc42 molecule participates in chronic inflammatory processes and oncogenesis [14, 15].

Cdc42 is one of the crucial molecules for intestinal epithelial cells (IECs) integrity and functioning, which has been proven in various *in vitro* studies, so far [16, 17]. Proper IEC polarity, interconnections, and differentiation are the basis of an adequate, uninterrupted intestinal epithelial barrier [18]. In one of the mice animal models it has been shown that Cdc42 deficiency affects Paneth cell differentiation leading to an impaired epithelial barrier [19]. Additionally, Cdc42 inactivation disrupts tight junctions between IEC by actin degradation [20].

Islet β-cells express Cdc42, indicating the significant role of this molecule in pancreatic function, particularly in insulin secretion and glucose homeostasis. Regarding localization in islet β-cells, Cdc42 is present in cytosol, specifically in insulin-secreted granules as well as attached to the plasma membrane. Therefore, it participates in β-cells cytoskeletal rearrangement and vesicle fusion taking part in glucose-stimulated insulin secretion (GSIS) [21, 22).

Considering the participation of the Cdc42 GTPase in various cellular physiological processes regarding gastrointestinal and pancreatic functions, the question arises about its role in pathological conditions, namely inflammatory bowel disease (IBD) and glucose metabolism impairment likewise hyperglycemia associated with diabetes mellitus. Revealing Cdc42 signaling pathways in the pathophysiology of these conditions is of great importance for potential prevention and better control of these disorders. This article critically reviewed the underlying mechanisms of Cdc42 regulation and signaling pathways involved in IBD, particularly associated with elevated blood glucose levels (hyperglycemia). Additionally, network analysis was performed to predict Cdc42 molecular interactions in relation to these pathological entities.

Regulation of Small Rho GTPases

Regulation of Rho GTPases signaling relies on two major groups of molecules called guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs). Their alternating action transfers GTPase from an active (GTP form) to an inactive form (GDP form) and vice versa, therefore making a small Rho GTPase as a "molecular switch", as previously mentioned. Hydrolysis of GTP and trasformation to GDP by enzyme GTPase is mediated by GAPs. On the other side, GEFs act as activators by mediating the phosphorylation from GDP-bound cytosol form, to GTP-bound active form of Rho GTPase. Once phosphorylated GTP form of GTPase can sequently bind to and activate some of its downstream effectors leading to various cellular functions [23]. As proteins that define small GTPase upstream signaling, GEFs action is induced by the cellular surface receptors. By the activation of external signals such as growth factors, integrins or cytokines, the surface receptors mediating GTPase signaling are activated, and considered as upstream initiators of Cdc42. These receptors belong to different families such as integrin receptors, glutamate receptors, G-protein coupled receptors (GPCR) or tyrosine kinases receptors (RTKs) [24]. The epidermal growth factor receptor (EGFR) belongs to a subclass of tyrosine kinase receptors (RTK) proteins. Activated by EGF it further activates MAPK increasing intestinal epithelial cells' regenerative capacity [17].

Another small Rho GTPase regulator is identified in the cytoplasm of the cells in the resting state. Namely, guanine nucleotide dissociation inhibitors (GDI) are responsible for maintaining intracytoplasmatic pool of small GTPases in the cell by binding to the GDP-bound GTPase and keeping it away from the cell membrane. When GDI dissociates from the GDP-bound form of GTPase, the latter will be attached to the cellular membrane where it is stimulated by the action of GEFs. Additional regulation of small GTPase signaling includes one of the post-translational modifications such as prenylation, phosphorylation, and ubiquitnation [25].

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Speaking from the perspective of Cdc42 signaling, when activated through one of the receptors (RTKs, GPCR, integrin receptors) Cdc42 acts on kinase as well as non-kinase targets in the cell. Non-kinase effectors of activated Cdc42 include partitioning-defective protein 6 (PAR6), Wiscott-Aldrich syndrome proteins (WASPs) and Q motif-containing GTPase-activating proteins (IQGAPs) which participate in maintaining cell polarity, cytoskeletal organization and vesicular transport [26]. On the other side, kinase effectors are divided into different subgroups like lipid kinases and protein kinases (Ser/Thr kinases and tyrosine kinases). Phosphatidylinositol 3-kinases (PI3-kinases) are lipid kinases that regulate cell proliferation, survival, and motility [27]. After activating Cdc42 they participate in mitogenic activity of the cell. Representatives of Ser/Thr kinases are p-21 activated kinase (PAK), mixed lineage kinase-3 (MLK3) and myotonic dystrophy kinase-related Cdc42-binding kinase (MRCK). Acting on these molecules, Cdc42 participates in cell-cycle regulation, transcriptional activation, and actin cytoskeleton arrangement [26]. Receptor mediated endocytosis is upregulated by activated Cdc42-associated kinase (ACK), in response to active Cdc42 stimulation (Figure 1). ACK is also known as non-receptor tyrosine kinase and for its action in promotion of oncogenesis [28]. Furthermore, the expression of ACK1 was significantly increased in colon epithelial cells of IBD patients compared to the control subjects and correlated with the activity of IBD [29]. The abovementioned molecules such as WASPs, PAR6, IQGAPs, PI3-kinase, PAK, MLK3, MRCK, and ACK could be considered downstream effectors of the Cdc42 molecule.

Cdc42 signaling in IBD

Inflammatory bowel disease (IBD) is a chronic intestinal disorder that typically includes two entities: Crohn's disease (CD) and ulcerative colitis (UC). Factors that participate in the pathophysiology of IBD include intestinal inflammation, gut dysbiosis, and disrupted epithelial barrier [30]. The exact cause of IBD is not yet elucidated, even though interplay between several contributing factors has been identified such as intestinal immune system, environmental factors, gut microbiome, and genetic susceptibility [31]. Disease progression is characterized by mitochondrial disfunction, autophagy dysregulation and increased inflammation [32].

Recently, the role of Cdc42 in the pathogenesis of IBD has been reported as well [33]. From the aspect of IBD pathobiology, it has been shown that Cdc42 in addition to other small GTPases participates in the dysregulation of the innate immune system (intestinal epithelial barrier, dendritic cells, macrophages) as well as the adaptive immune system (cellular and humoral immunity) [34-36]. Experimental dextran sulfate sodium (DSS) induced colitis in mice has been found to be ameliorated by decreased expression of Cdc42 in combination with increased RhoB

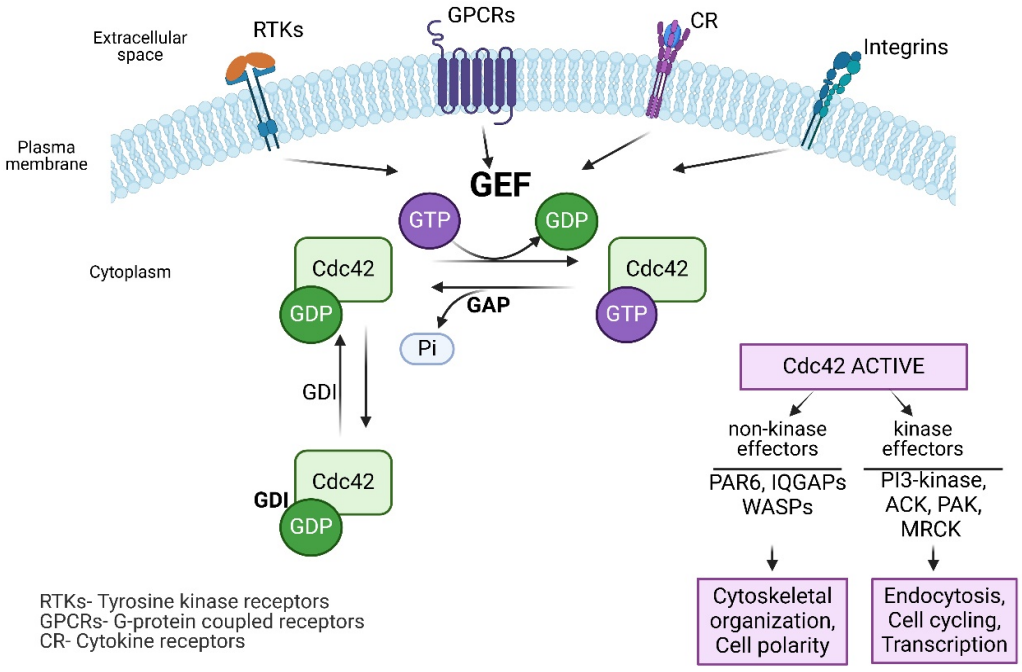

Figure 1: Regulation of Cdc42 molecular signaling. Major regulators of Cdc42 cellular functions are guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs), in addition to guanine nucleotide dissociation inhibitors (GDIs). Depending on the regulators, Cdc42 is present in the cell in active GTP-binding form (activated by GEFs), inactive GDP-binding form (mediated by GAPs), or as intracytoplasmatic poll binded to GDIs. Growth factors, cytokines and integrins, by the transmembrane signaling stimulate GEFs leading to Cdc42 activation. Once activated, Cdc42 controls various cellular functions, mainly by regulation of its downstream effectors.

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expression regulated by miRNA21 [37]. Maintenance of the functional intestinal epithelial barrier depends on adequate IEC interconnections, especially tight junctions-TJ. F-actin degradation and thereafter TJ interruption appear as a sequel of Cdc42 inactivation, acting together detrimental to the intestinal epithelial barrier [20]. Another *in vitro* study on mice was designed to investigate inflammatory markers in an experimental model of IBD. Results of these experiments showed increased levels of IL-10, IFN-γ, IL-4, and TNF-α in the IBD groups, while this effect was abolished by the Cdc42 treatment [38]. The latter indicates the role of Cdc42 in the prevention of inflammation in the intestines by still not elucidated mechanism. One of the potential modes of action of Cdc42 regarding this matter could be the regulation of the macrophages' kinetics and migration by modulating some of the pathways responsible for their actin cytoskeleton organization and function (Table 1).

One of the latest studies on LPS-treated human Caco-2 cell line reported that inhibition of the Cdc42/Janus kinase (JNK) pathway is responsible for anti-inflammatory, anti-oxidative, and anti-apoptotic properties of paeoniflorin (PF), an active terpene glycoside, suggested it to be a novel Cdc42 inhibitor [39, 40]. In addition, from the perspective of human studies, Cdc42 was subjected as a biomarker for the progression of ulcerative colitis, since the correlation has been found between reaction to infliximab and levels of Cdc42 expression [41]. Another study on humans showed reduced expression of Cdc42 in colon IECs of IBD patients in comparison to controls [42].

Role of dendritic cells and macrophages

Dendritic cells (DCs), macrophages and neutrophils represent important regulators of immune response in the gastrointestinal tract in both physiological and pathophysiological conditions. Dendritic cells and macrophages have antigen-presenting properties, even though macrophages participate more in phagocytosis of apoptotic cells and pathogens [43]. It has been suggested that inflammatory cells like macrophages and T-lymphocytes may contribute to epithelial regeneration, and therefore modulation of their function might be one of the strategies for therapeutical interventions [44, 45].

As reported in the mice experimental model, deficiency of Cdc42 in T cells consequently led to an aggravated form of colitis [46]. Furthermore, Cdc42 has been shown to positively regulate phagocytosis by macrophages, together with Ras-related C3 botulinum toxin substrate 1 (Rac1) [47]. Stimulation of macrophage phagocytosis by Cdc42 is mediated through the pathway: WASP- actin-related protein 2/3 complex (Arp 2/3) [48]. Apoptotic cells engulfing is regulated by the action of integrins and stimulated by Cdc42, but negatively regulated by other Rho GTPases [49].

Table 1: Cdc42 signaling in IBD as presented in animal experimental models and human studies.

Study type	Effect	Reference
DSS colitis model in mice	Ameliorated colitis by Cdc42	(37)
TNBS IBD model in mice	Reduced inflammation by Cdc42	(38)
Experimental model in mutant mice	IEC hyperproliferation and increased crypt depth in response to Cdc42 deletion	(19)
Experimental study in aged mice	Improved crypt regeneration by Wnt pathway in response to Cdc42 inhibition	(57)
Mouse models of colitis	Exacerbation of colitis due to Cdc42 deficiency in T-cells	(46)
Human study in IBD patients	Cdc42 as a biomarker for UC progression	(41)
Human study in IBD patients	Decreased Cdc42 expression in colon IEC of IBD patients vs controls	(42)
Human study in IBD patients	Increased ACK1 expression in colon epithelial cells with inflammation and dysplasia vs controls	(29)

ACK1- Activated Cdc42 kinase1; DSS, dextran sodium sulfate; IBD-inflammatory bowel disease; IEC-Intestinal epithelial cell; TNBS, Trinitrobenzene sulfonic acid; UC-Ulcerative colitis

Actions of conventional DCs (cDCs) and plasmacytoid DCs (pDCs) have been reported in the development of IBD [50, 51]. Namely, activated by the luminal contents through disrupted epithelial barrier cDCs produce proinflammatory cytokines like IL-12 and IL-23 and activate Th1 cells and therefore promote inflammation of the intestinal wall. In addition, according to some authors in patients with active forms of IBD, pDCs migrate to secondary lymphoid organs and produce inflammation by releasing following cytokines: TNF-α, IL-6 and IL-8 [52, 53]. Dynamic relation between Rho GTPases including Cdc42, Rac and Rho are necessary for proper DCs function in terms of antigen presentation at first place, as well as their migration [54]. Experimental DSS-induced colitis was aggravated by the inflammatory reaction induced by excessive stimulation of cDCs. cDCs activation was found to be mediated by Toll-like receptor 4 (TLR4) in response to bacterial lipopolysaccharide (LPS) [55]. Furthermore, endocytosis in DCs has been found to be dependent on Cdc42. According to the results reported by Garret et al. [56] active form of Cdc42 binds to WASP or N-WASP, which in the next step bind to Arp 2/3 complex leading to acting filament formation and therefore producing

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sufficient force to form membrane protrusions and finally phagosome [56]. Taking into consideration that DCs have pro-inflammatory effects in the IBD experimental conditions, it could be considered that modulation of their function and pathways they participate in could be potential target for IBD therapeutical interventions.

Role of Intestinal epithelial stem cells

In the intestine, Cdc42 has been shown to have an important role in maintaining the function of intestinal epithelial stem cells (IESC), by regulating their survival and differentiation. Cdc42 is also implicated in IESC division, as well [18]. Lgr-5 (Leucine-rich repeat-containing G protein-coupled receptor 5) positive (Lgr5+) IESCs regulate proper intestine cell dynamics [57]. Recent report showed that Cdc42 is necessary for Lgr-5+ IESCs proliferation to progenitors and therefore for mucosal regeneration. Otherwise, Cdc42 reduction could make intestinal epithelium more prone to damage and therefore for IBD-associated intestinal wall inflammation [42]. Furthermore, the authors reported IESC hyperproliferation and crypt hyperplasia in response to Cdc42 deletion [19]. Cdc42 levels were increased in aged IESCs leading to impaired regenerative capacity of the mucosal barrier. According to this study, the pharmacological modulation of Cdc42 activity in aged mice, by Cdc42 activity specific inhibitor (CASIN), improved crypt regenerative capacities. The Canonical Wnt signaling pathway was suggested as an upstream regulator of Cdc42 activity in this study, proven by a Wnt ligand (Wnt3a) treatment [57].

When talking about leucine-rich repeat-containing G-protein coupled receptors (Lgr 4-6), it is worth mentioning that they typically function associated with specific ligands named R-spondins (Rspo), regulating stem cell survival and oncogenesis [58, 59]. It has been reported that R-spondin1 acts as a specific mitogen for small intestinal and colon epithelial cells in mice, without affecting the proliferation of goblet and Paneth cells [58]. R-spondins have been identified as upstream (positive) regulators of the Wnt-β catenin pathway. Regulation of this signaling pathway (Wnt-β catenin) has been shown to control the growth of gastrointestinal epithelial cells and to contribute to the pathophysiology of carcinogenesis [60]. IQ motif containing GTPase-activating protein 1 (IQGAP1) was shown to mediate R-spondin-Lgr4 interaction via the Wnt pathway [61]. Additionally, Lgr4 is usually co-expressed with Lgr5 and Lgr6 to regulate the crypts' stem cell physiology in the intestinal tract [62]. Furthermore, according to one experimental study on mice, Lgr4 maintains the physiology of intestinal epithelium, especially by protecting it from IBD, through the Wnt-β-catenin pathway [63]. In addition, R-spondin3 plays a crucial role in regenerating the colon epithelium, as proven in the DSS-induced colitis model in mice. This study suggests that during the regeneration process, Rspo3 activates Wnt signaling by binding to Lgr4 receptors, leading to new crypt formation [64]. It remains to be elucidated whether and to what extent R-spondin-Lgr interaction plays a role in the pathogenesis of IBD in hyperglycemic conditions.

ACK1- Activated Cdc42 kinase1; DSS, dextran sodium sulfate; IBD-inflammatory bowel disease; IEC-Intestinal epithelial cell; TNBS, Trinitrobenzene sulfonic acid; UC-Ulcerative colitis

Cdc42 signaling in hyperglycemia

It has been reported that Cdc42 participates in different stages of insulin secretion (the granules mobilization, and exocytosis of the granules) as well as the proliferation of pancreatic β cells [65]. Pancreatic β cell proliferation is regulated by Cdc42-PAK1 and Cdc42-cyclinD1 pathways. Additionally, mobilization of the granules is regulated by the Cdc42 and its downstream effectors like: N-WASP-Arp2/3 in one or PAK1/MEK/ERK in another signaling pathway [66-69]. According to this, it can be said that PAK1 is one of the Cdc42 downstream effectors contributing to insulin release from the β cells. From the pathophysiological point of view, inhibition of PAK1 by Cdc42 can impair insulin secretion and consequently contribute to insulin resistance (IR).

Aside from its effects in the pathophysiology of insulin resistance, Cdc42 is involved in other conditions associated with diabetes mellitus (DM), such as diabetic nephropathy (DN). Cdc42 contributes to DN by being a part of pathways controlling different processes such as: podocytes injury, podocytes migration, mesangial cells injury, glomerulosclerosis and tubular fibrosis. In these pathways Cdc42 interacts with one of the following molecules: TGF-β, SRGAP2a (SLIT-ROBO ρGTPase-activating protein 2a) or PI3K (phosphoinositide3-kinase) [70-73]. According to the recent finding Cdc42 expression was high in podocytes of diabetic mice proven with diabetic nephropathy, leading to podocytes apoptosis [74].

The potential participation of Cdc42 signaling in the therapy of DM is still to be elucidated. Identifying and later modifying key Cdc42 regulatory patterns in the pathogenesis of DM would help improve current therapy algorithms and hopefully better control of hyperglycemia. Even though insulin has wide therapeutical application in clinical settings, it was suggested that it increases the risk of colorectal and pancreatic cancer [75, 76]. Aside from its beneficial effects in blood glucose control, semaglutide (a GLP-1 agonist) therapy bears the risks of development of acute pancreatitis, acute kidney injury or diabetic retinopathy [77, 78].

Aside from the fact that Cdc42 has already been discussed separately in IBD and hyperglycemic conditions, there is a lack of scientific results reporting the nature of Cdc42 signaling in IBD under hyperglycemic conditions. Therefore, by reviewing the major regulators of Cdc42 (GEFs and GAPs) and their function and contribution to IBD and hyperglycemia we aimed to identify their mutual interactions.

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Cdc42 regulation by major GEFs

Two different families of GEFs were identified in upstream control of the small GTPases: diffuse B-cell lymphoma (DBL) family proteins and dedicator of cytokinesis (DOCK) proteins [24].

DOCK8 is a specific GEF for Cdc42, according to the study performed by Harada et al [79]. They provided scientific evidence from the functional and biochemical perspective for DOCK8 as a regulator of DCs migration, by controlling Cdc42. This could be considered important from the immunological point of view since DCs initiate adaptive immunity by migration from the peripheral tissues into the lymph nodes to stimulate T-lymphocytes [80]. Furthermore, it is shown that DOCK8 binds to WASP and ARP2/3 to mediate a T- cell response by interconnecting T-cell receptor with actin cytoskeleton [81]. As recently reported, DCs also participate in the initiation and progression of inflammatory response in IBD [82]. The data regarding Cdc42 upregulation in terms of IBD are very scarce. Whether, and if, to which extent DOCK8 participates in Cdc42 signaling in IBD patients in hyperglycemic conditions is yet to be explored.

Cdc42 regulation by major GAPs

GAPs participate in Cdc42 regulation by binding to it and stimulating hydrolysis of GTP through activation of Cdc42 GTPase [83]. Rho GTPase activating protein 31 (Arhgap31) is GAP recently reported to mediate Cdc42 signaling in intestinal stem cells. This study revealed that this molecule is a constituent of the pathway: DLG1-Arhgap31-Cdc42 which is of great importance in the response of intestinal stem cells to Wnt signaling. Namely, in response to intestinal tissue damage of different origins (inflammation, irradiation, resection), an increase in Wnt signaling occurs, leading to regeneration, inducing migration and proliferation of intestinal stem cells. This effect is achieved by activation of DLG1, which further increases the activity of Arhgap31, inhibiting Cdc42 and finally stimulating intestinal stem cell proliferation. In this particular pathway, Cdc42 is considered a mediator of non-canonocal Wnt signaling. Herein it is worth mentioning that, non-canonical Wnt pathways are non dependent of β-catenin, while canonical Wnt signaling is characterized by β-catenin as a mediator that translocates from the cytoplasm to the nucleus where it regulates genes expression [84].

From the aspect of IBD, a transient increase in Wnt signaling is considered of special importance, since it promotes regeneration. Furthermore, in the conditions of increased Wnt signaling, Discs large 1 (DLG1) has a significant role in intestinal stem cell survival [85].

Network analysis of Cdc42 in relation to IBD and hyperglycemia

In order to get better insight into Cdc42 molecule interactions we performed in silico analysis using The SIGnaling Network Open Resource (SIGNOR 3.0; www.signor.uniroma2.it). The SIGNOR 3.0 analysis revealed interactions of Cdc42 with various up-stream and down-stream regulators as presented in Figure 2. Considering our area of interest (IBD and Hyperglycemia) we selected major regulators of Cdc42 from here, for further analysis using Network analyst (www.networkanalyst.ca).

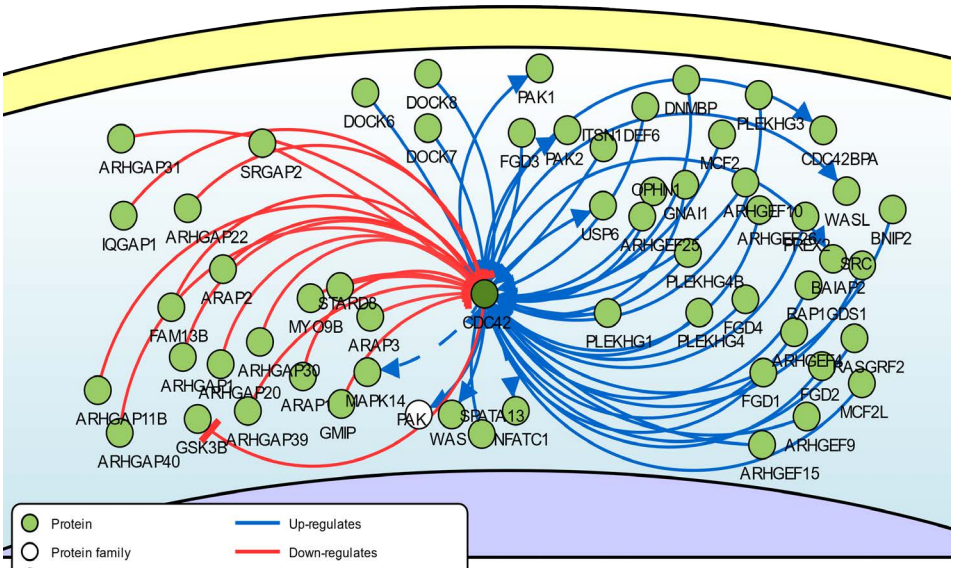

Figure 2: The SIGnaling Network Open Resource (SIGNOR 3.0) analysis reveals various upstream-regulators (blue lines) and downstream-regulators (red lines) of Cdc42.

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We used Network analyst to further analyze the interactions of Cdc42 with other proteins, so-called protein-protein interactions (PPI). Gene input names were chosen according to the previous SIGNOR analysis, as well as the literature review of genes involved in Cdc42 signaling in IBD, intestinal epithelial regeneration, hyperglycemia, diabetes mellitus, insulin secretion, in addition to major GEFs and GAPs of Cdc42 related to IBD. The input list for PPI network analysis, using IMEx interactome, included the following genes: Cdc42, DOCK8, IQGAP1, MAP4K3, ACK1, LGR4, LGR5, RSPO3, RSPO1, WNT, CTNNB1 (β-catenin), PAK1, ARHGAP31, DLG1, CCND1 (CyclinD1), N-WASP, ARP2/3 (Figure 3.). The outcomes from the network analysis revealed direct interactions between Cdc42 and the following proteins: ITSN1, DOCK8, CDH1, RAC1, IQGAP1, CDKN1A, ELAVL1, GRB2, TGFBR2, PAK1, RUVBL1, PRKCA, GOPC, PIK3R1, UBC. According to the node size, Cdc42 showed the highest connectivity with CTNNB1 (β-catenin), even though this interaction was not direct. Predicted mediators between Cdc42 and CTNNB1 (β-catenin) were as follows: UBC, GOPC, PRKCA, PIK3R1, RUVBL1, TGFBR2, and CDH1 (Figure 3.).

Cdc42 regulation by transcription factors (TFs)

The regenerative capacity of the intestinal epithelium is of great importance when it comes to tissue damage for different reasons, even though the exact underlying mechanisms remain unknown. It has been proposed that intestinal epithelial renewal, from the aspect of genotoxic injury, is mediated by the Cdc42-Mitogen-activated protein kinase (MAPK) pathway, examined in a mice experimental model [86]. Namely, Cdc42 engaging with EGFR was shown to be sufficient for MAPK activation, stimulated by Epidermal growth factor (EGF). In addition to the previously mentioned canonical Wnt-signaling, the EGF-mediated pathway is implicated in intestinal stem cell regeneration and survival [87, 88]. Whether and to which extent these molecules are related to IBD is yet to be elucidated, especially in hyperglycemic conditions.

The network analysis using Networkanalyst.ca revealed the association of input genes related to Cdc42 signaling in IBD and hyperglycemia with various transcription factors (Figure 4.). To predict TF-gene interactions in the above-mentioned conditions we used the JASPAR database with

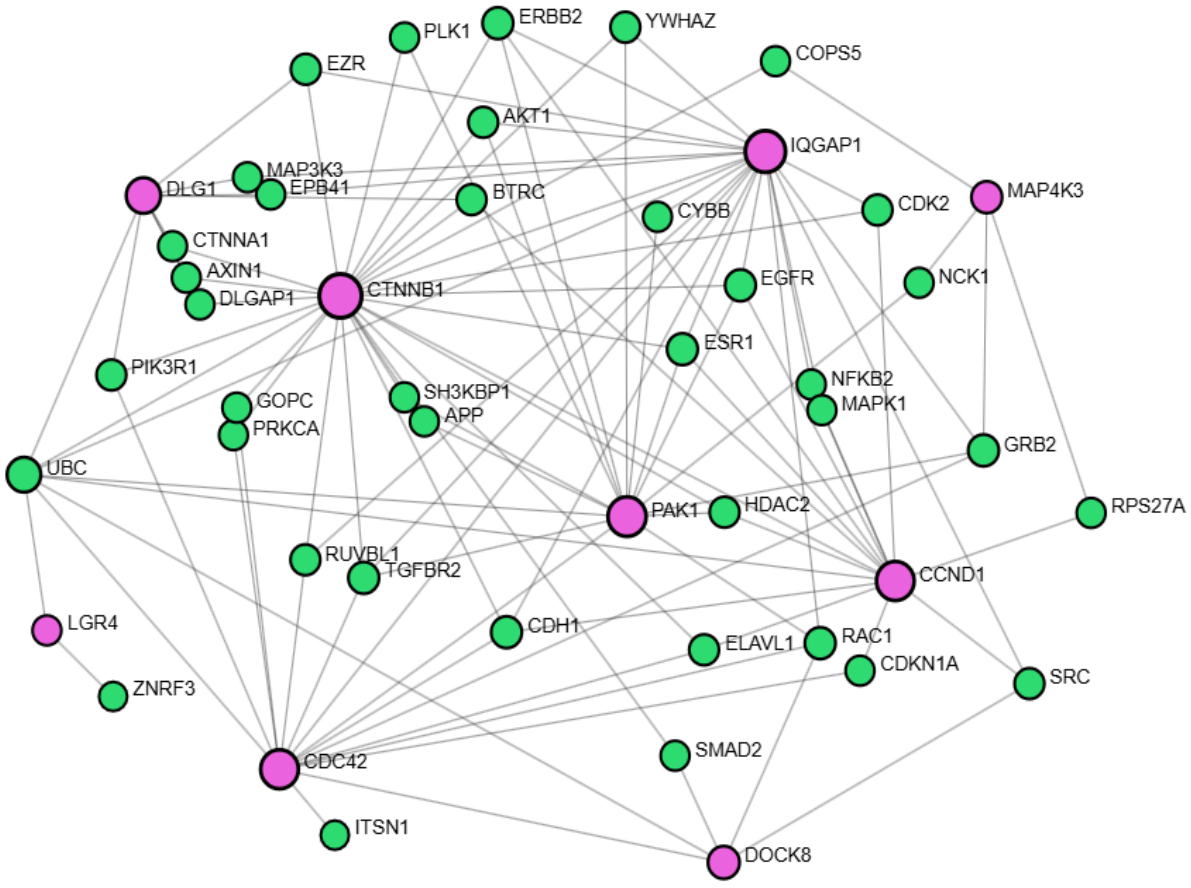

Figure 3: Network analysis for the Cdc42 protein-protein interactions (PPI) using IMEx interactome. Rose circles (input proteins), green circles (output proteins). Greater node size represents increased connectivity.

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the following input genes: Cdc42, DOCK8, IQGAP1, MAP4K3, ACK1, LGR4, LGR5, RSPO3, RSPO1, WNT, CTNNB1 (β-catenin), PAK1, ARHGAP31, DLG1, CCND1 (CyclinD1), N-WASP, ARP2/3, UBC, ELAVL1, RAC1, GRB2.

Interferences drawn from the network analysis indicate that TFs including TFAP2A, NFIC, E2F1, PAX2 and CREB1 exert control over Cdc42 expression by regulating following genes: LGR4, CCND1, RSPO3 and DOCK8, with a betweenness (TFs influence on gene) of 20.34; 6.22; 93.75; 34.88 and 82.27 respectively. Additionally, Cdc42 may be regulated by GRB2, CCND1, ARHGAP31 controlled by the following TFs: E2F6 (betweenness 7.52), HOXA5 (betweenness 30.59) and FOXC1 (betweenness 463.47). The notable high value of betweenness for FOXC1 indicates its important influence on the gene, as presented by the size of the square on the graph (Figure 4).

DOCK8 has been shown to be one of the majors GEFs of Cdc42. According to the predictions made by network analysis, it may interact with RSPO1 or RSPO3, controlled by TFs such as IRF2 or PAX2. IQGAP1 is one of the proposed down-stream molecules that regulates Cdc42 signaling. Predicted genes to interact with IQGAP1 according to the network analysis might be CCND1 and GRB2 controlled by the following TFs: KLF5, EGR1 and E2F6.

Despite all the predictions made by the network analysis, TF-gene interactions of interest warrant further investigation in the in vitro models. That approach will help to get better insight in the nature of different TF-gene interactions in terms of up- and down-regulation.

Conclusion

Considering the variable presentations of IBD among patients, the need for an optimal therapeutic strategy, aside from the conventional one typically focused on the symptoms, arises. Identifying important target molecules will help in potential translational application in the clinical setting by modifying them to achieve better control of IBD by preventing its progression. Potential strategy could refer to the stimulation or inhibition of the key regulators of the Cdc42 pathway. In addition, molecules essential for intestinal epithelium recovery, likewise R-spondin3 might be considered for pharmaceutical modulation on some of the key regulators of the molecular network.

Since literature data regarding Cdc42 signaling in hyperglycemic conditions are very limited, in silico analysis performed in this study revealed potential interactions regarding this matter. Network analysis highlights various genes included in Cdc42 signaling, related to IBD and hyperglycemia, and their regulation by transcription factors.

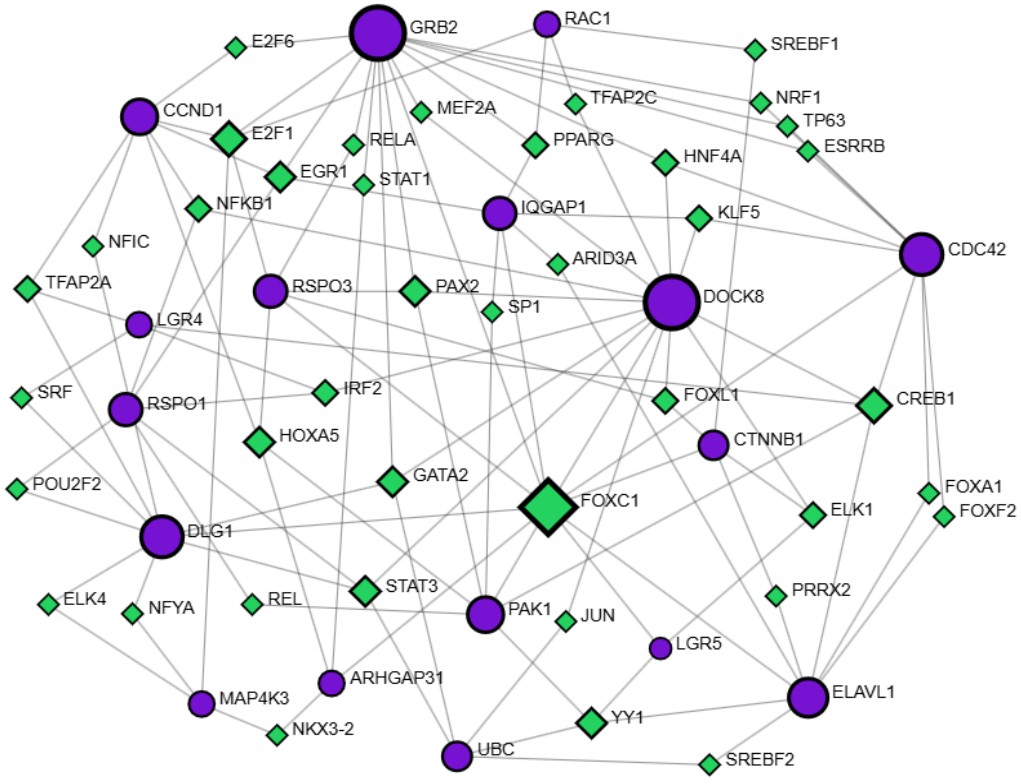

Figure 4: Transcription factor-gene interactions (related to Cdc42 signaling) network analysis using the JASPAR database. Purple circles (input genes); green squares (output transcription factors).

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According to the resulting interactions, potential Cdc42 pathways of special importance in IBD might be identified. Since our conducted study is based on the prediction, future investigations in vitro or in vivo conditions are needed to get a better insight in the pattern of the Cdc42 signaling and regulation.

Key points

- Cdc42 belongs to a small-GTPase family, as a "molecular switch" which transforms between inactive (GDP-binding) and active (GTP-binding) state.
- Cdc42 regulates intestinal epithelial cells' polarity and interconnections leading to the proper function of the intestinal epithelial barrier.
- Cdc42 regulates glucose-stimulated insulin secretion as well as differentiation of pancreatic β-cells.
- Different experimental studies showed impaired Cdc42 signaling in IBD and hyperglicemia, even though there is lack of evidence about Cdc42 molecular pathway in IBD during hyperglycemic conditions.
- Regulation of Cdc42 molecular signaling is based on the major GEFs and GAPs. DOCK8 is one of the major GEFs leading to activation of Cdc42, while Rho GTPase activating protein 31 (Arhgap31) inactivates it as a GAP.
- Wnt pathway was shown to regulate Cdc42 signaling in intestinal epithelial stem cells.
- Different upstream (DOCK6, DOCK7, DOCK8, PAK1) and downstream (Arhgap31, IQGAP1, MYO9B) regulators of Cdc42 were identified using SIGNO3.0 analysis.
- Network analysis performed in this study revealed interactions between various genes related to both IBD and hyperglycemia.
- According to in silco analysis transcription factors included in Cdc42 signaling related to IBD and hyperglycemia has been suggested.
- Predicted molecular interactions of Cdc42 signaling need approval by future in vitro or in vivo experimental studies aimed to identify specific target for pharmacological interventions related to IBD and hyperglycemic conditions.

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