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Review

GIT2—A keystone in ageing and age-related disease

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ABSTRACT

Since its discovery, G protein-coupled receptor kinase-interacting protein 2, GIT2, and its family member, GIT1, have received considerable interest concerning their potential key roles in regulating multiple inter-connected physiological and pathophysiological processes. GIT2 was first identified as a multifunctional protein that is recruited to G protein-coupled receptors (GPCRs) during the process of receptor internalization. Recent findings have demonstrated that perhaps one of the most important effects of GIT2 in physiology concerns its role in controlling multiple aspects of the complex ageing process. Ageing can be considered the most prevalent pathophysiological condition in humans, affecting all tissue systems and acting as a driving force for many common and intractable disorders. The ageing process involves a complex interplay among various deleterious activities that profoundly disrupt the body's ability to cope with damage, thus increasing susceptibility to pathophysiologies such as neurodegeneration, central obesity, osteoporosis, type 2 diabetes mellitus and atherosclerosis. The biological systems that control ageing appear to function as a series of interconnected complex networks. The inter-communication among multiple lower-complexity signaling systems within the global ageing networks is likely coordinated internally by keystones or hubs, which regulate responses to dynamic molecular events through protein-protein interactions with multiple distinct partners. Multiple lines of research have suggested that GIT2 may act as one of these network coordinators in the ageing process. Identifying and targeting keystones, such as GIT2, is thus an important approach in our understanding of, and eventual ability to, medically ameliorate or interdict age-related progressive cellular and tissue damage.

1. Introduction

Mammalian GIT1 was first identified as a binding partner for G protein-coupled receptor kinases (GRKs), and thus named GRK-interacting protein 1 (GIT1) (Premont et al., 1998). GIT1 and GIT2 comprise the GIT protein family, which share enzymatic function as GTPase-activating proteins (GAPs) for the ADP-ribosylation factor (Arf) small GTP-binding proteins (Premont et al., 1998; Vitale et al., 2000). GIT proteins function to limit the activity of Arf proteins, and are members of the larger family of ArfGAPs (Kahn et al., 2008). Arf proteins have no intrinsic GTPase activity, and thus require GAPs to convert the GTP bound to active Arf to GDP, causing deactivation (Randazzo et al., 1994). Both GIT proteins were originally identified as regulators of GPCR internalization through the influence they exert on the Arf GTP-binding proteins (Claing et al., 2000; Premont et al., 1998; Vitale et al., 2000). Purified GIT proteins are linked functionally to plasma membrane protein Arf6 (Claing et al., 2000; Di Cesare et al., 2000; Jones et al., 2009; Meyer et al., 2006; Miura et al., 2009), but inactivate all subtypes of Arf proteins (Vitale et al., 2000).

GIT proteins and their primary interaction partners, the PIX (PAK

Abbreviations: AD, Alzheimer's disease; ArfGAP, ADP-ribosylation factor GTPase-activating proteins; AT, adipose tissue; ATM, Ataxia Telangiectasia Mutated; ATP, adenosine triphosphate; BRCA1, breast cancer type 1 susceptibility protein; CNS, central nervous system; DDR, DNA damage response; DSB, double-strand break; EGF, endothelial growth factor; GIT2, G protein coupled receptor kinase interacting protein 2; GPCR, G protein-coupled receptor; Ins, insulin; IGF1, insulin growth factor 1; IL, interleukin; NF-κB, nuclear factor NF-kappa-B; OB, osteoblast; OC, osteoclast; PARP, poly (ADP-ribose) polymerase; PIX, PAK (p21-activated kinase) interacting exchange factor; RANK, receptor activator of nuclear factor kappa B; ROS, reactive oxygen species; RUSC2, RUN and SH3 Domain Containing 2; T2DM, Type 2 Diabetes Mellitus; TLR, Toll-like receptor; TNFα, tumor necrosis factor-alpha; WT, wild-type

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(p21-activated kinase)-interacting exchange factor) proteins, can function together as signaling scaffolding proteins with their multiple domains binding to many protein partners (Zhou et al., 2016). The most well described binding partners of GIT proteins are p21-activated kinase-interacting exchange factors α-PIX and β-PIX (Bagrodia et al., 1998; Premont et al., 2000, 2004; Zhao et al., 2000). GIT proteins act as part of this scaffold complex to link signaling molecules to distinct sites of action in the cell and within many distinct signaling networks. Over 100 GIT-associated proteins and dozens of direct interactors, many of which have been first identified in the brain, have been described (Table S1) including liprin-α, piccolo, and huntingtin (Hoefen and Berk, 2006; Zhou et al., 2016). GIT proteins have been implicated in the regulation of cognition, where loss of GIT1 resulted in severe learning and memory deficiencies in three distinct murine knockout models (Hong and Mah, 2015; Menon et al., 2010; Schmalzigaug et al., 2009a; Won et al., 2011), and microcephaly due to a neuron size reduction (Hong and Mah, 2015), while GIT2-KO mice exhibit anxiety-like behavior and advanced ageing (Lu et al., 2015; Schmalzigaug et al., 2009b).

The GIT proteins have been implicated in multiple cellular processes, including cell migration (Zhao et al., 2000), dendritic spine formation (Zhang et al., 2003, 2005), T-cell activation (Phee et al., 2005), huntingtin aggregation (Goehler et al., 2004) and centrosome dynamics (Zhao et al., 2005). Brain tissues from Huntington's disease patients have been shown to display the accumulation of a C-terminal proteolytic fragment of GIT1 (Goehler et al., 2004). GIT1 localizes in both pre- and post-synaptic terminals in hippocampal neurons (Zhang et al., 2003) and downregulation or mislocalization of GIT1 leads to disrupted dendritic spine and synapse formation (Zhang et al., 2003, 2005). Additionally, GIT1 facilitates AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptor targeting in primary neurons of the hippocampus (Ko et al., 2003) and mediates ephrin B signaling during spine formation (Segura et al., 2007). In contrast, less is known about the neuronal functions of GIT2, despite the large expression overlap between GIT1 and GIT2 throughout the brain (Schmalzigaug et al., 2007b).

Mammals express GIT1 and GIT2, whereas zebrafish express three GIT proteins, since they have 2 GIT2 genes (*git2a* and *git2b*) (Yu et al., 2011). While GIT1 has only two splice variants in humans and mice, GIT2 undergoes extensive alternative splicing (Premont et al., 2000). GIT1 and GIT2 share a conserved domain architecture, including the N-terminal zinc finger ArfGAP domain, three Ankyrin repeats, a Spa2-homology domain/Src homology 2 domain-containing transforming protein D (SHD), a coiled-coil (CC) domain, a poorly conserved linker region and a focal adhesion targeting (FAT) domain (Fig. 1A&B) (Zhou et al., 2016). The α-PIX and β-PIX interacting partners bind to the SHD domain. The coiled-coil domain allows dimerization of GIT proteins through formation of a parallel coiled-coil (Premont et al., 2004; Schlenker and Rittinger, 2009). The FAT domain acts as the binding site for the focal adhesion adaptor protein paxillin (Schmalzigaug et al., 2007a; Zhang et al., 2008). GIT2-short is a truncated variant of GIT2 that is missing the FAT domain (Fig. 1C) and is highly expressed in immune cells. The importance of GIT2 splicing remains unclear, though GIT2-short displays an inability to bind to paxillin in the same manner as GIT2 (Premont et al., 2000) and both GIT1 and GIT2 are able to regulate Arf6-dependent GPCR sequestration. The direct comparison between GIT1 and GIT2 suggests that GIT2 binds to paxillin with much lower affinity than GIT1 (Premont et al., 2000). Another difference is that GIT1 tyrosine phosphorylation is unaffected by cell adhesion, while GIT2 is transiently phosphorylated during attachment (Shikata et al., 2003). The high homology seen between GIT1 and GIT2, both in structure and function as well as the strong homo- and hetero-dimerization of these proteins, suggests the presence of some redundancy *in vivo*, implying that these exert different functions. To analyze the individual GIT proteins in a cellular context, Schmalzigaug et al. investigated tissue- and cell-specific expression patterns (Schmalzigaug et al., 2007b). While their research in mice confirmed the broad distribution of the two GIT genes seen in human and rat, it also revealed underlying expression patterns. GIT2 appears to be nearly ubiquitously expressed, whereas GIT1 expression distribution is much more restricted. Both GIT1 and GIT2 are co-expressed throughout most of the brain, except for the cerebellum, where only GIT2 can be found in the granule cells. While GIT1 expression is restricted mainly to the vasculature in liver and lung, GIT2 is expressed in most cell types (Schmalzigaug et al., 2007b). Furthermore, GIT1 and GIT2 genes are regulated in a cell maturation-dependent manner in testes, where GIT2 expression is turned on in early-stage spermatogonia cells but is turned off as these cells mature; GIT1 expression shows the opposite pattern. This suggests a developmental shift in expression between the two isoforms. Even though both GIT1 and GIT2 expression is prominent in testes, deficiency in these genes does not cause male infertility, indicating that neither GIT gene is absolutely required for normal sperm development and function (Schmalzigaug et al., 2007b). Taken together, while both exert functions in the brain, GIT1 is mainly involved in brain development and GIT2 possibly has a more prominent role in neurodegeneration due to ageing (Goehler et al., 2004; Hong and Mah, 2015; Lu et al., 2015).

1.1. Molecular mechanisms of ageing

Ageing is one of the most complex and interconnected biological processes known, characterized by a progressive loss of physiological integrity that leads to impaired functionality and increased vulnerability to morbidity and in the end mortality (Lopez-Otin et al., 2013). This process also represents one of the highest risk factors for many major human disorders, *i.e.* neurodegeneration, osteoporosis, and Type

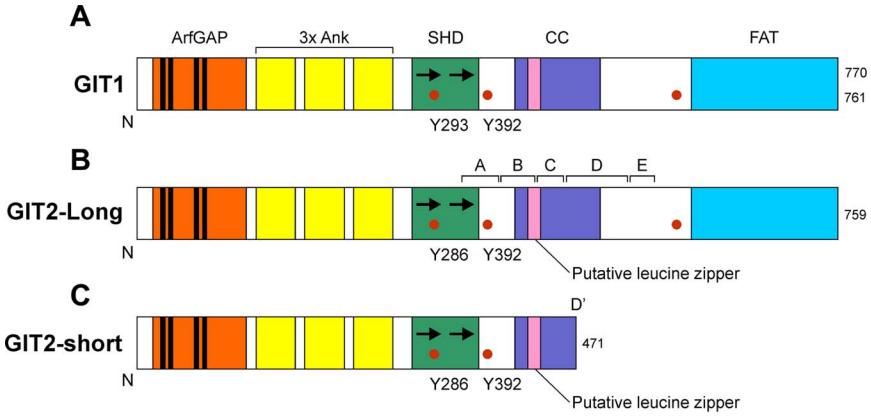

Fig. 1. *Domain-based structure of GIT family proteins.* GIT1 (A) and GIT2 (B) share a common amino-terminal (N) ArfGAP (ADP ribosylation factor (Arf) GTPase-activating protein) domain, three ankyrin repeats (3x Ank), and the Spa homology domain (SHD) – indicated with two Spa2-like repeats – a coiled-coil (CC) dimerization domain and a C-terminal focal adhesion-targeting (FAT) domain. The ArfGAP domains facilitate the GTP hydrolysis on Arf1 and Arf6; Ankyrin repeats promote the integration and stabilization of multiple transmembrane proteins; the SHD regions mediates PIX, FAK, MEK, Piccolo binding; the CC domain is likely associated with regulating transcriptional functions; the FAT domain mediates localized targeting to plasma membrane-bound integrin complexes. In contrast to GIT1, GIT2 has been shown to exist as multiple isoforms, which are produced due to alternative splicing of five internal in-frame regions (indicated with A, B, C, D, E). Additionally, a distinct exon (D') exists which leads to GIT2-short (C), a truncated isoform where the FAT domain is absent. For GIT1, alternative splicing of a single exon can occur at the start of the SHD. Prominent post-translational modification phosphorylation sites (tyrosine – Y) are shown in red.