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The mTOR independent function of Tsc1 and FNIPs

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Abstract

New roles for Tsc1 and FNIP1/2 as regulators of the molecular chaperone Hsp90 were recently identified, demonstrating a broader cellular impact outside of AMPK/mTOR signaling. In studying the function of these proteins we must take a holistic view of the cell, instead of maintaining our focus on a single pathway.

Keywords

Birt-Hogg-Dubé syndrome; Tuberous Sclerosis Complex; Hsp90; co-chaperone; mTOR

The molecular chaperone Hsp90

The molecular chaperone Hsp90 is a key element of the cellular proteostatic machinery and is essential for the maturation and activation of an array of ‘client’ proteins. Hsp90 clients are diverse and encompass various kinases, transcription factors, including steroid hormone receptors, and many others (<https://www.picard.ch/downloads/Hsp90interactors.pdf>). These clients are involved in cellular processes including, but not limited to, cell cycle control, proliferation, cell signaling, and gene expression [1].

Hsp90 function is coupled to its ability to hydrolyze ATP and is intricately regulated by a group of proteins known as co-chaperones. Co-chaperones affect the dynamics of the cycle of Hsp90 conformational changes and in many cases help load clients to the chaperone machinery [1]. We recently identified the proteins FNIP1, FNIP2 (collectively FNIPs) and Tsc1 as new co-chaperones of Hsp90 [2, 3].

Folliculin-interacting proteins-1/2 (FNIP1/2) were originally identified as integral partners of the tumor suppressor folliculin (FLCN), mutations in which cause Birt-Hogg-gDubé

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(BHD) syndrome [4]. The clinical manifestations of BHD syndrome share many similarities with those of Tuberous Sclerosis Complex (TSC), which is caused by mutations in either Tsc1 or Tsc2 [5].

The initial characterization of FNIP1/2 and Tsc1 through their interaction and protection effects for FLCN and Tsc2, respectively, led to the identification of roles linked to mTOR signaling. Subsequent work on these proteins has focused nearly exclusively on mTOR-associated effects and ignored early and growing evidence that FNIP1/2 and Tsc1 have additional functions. We call for a broadening of the view in this field in order to better appreciate the functions of these proteins.

FNIP1/2 regulate AMPK via FLCN

The first identified role for FNIP1 and FNIP2 was to stabilize the tumor suppressor FLCN. Early reports searching for the function of FNIP1 and FLCN identified an interaction with AMPK, a nutrient sensing kinase that regulates the mTOR pathway. The vast majority of the subsequent work in the field focused narrowly on a FNIP1-AMPK relationship by examining FNIP1 loss of function phenotypes in muscle fiber type specification and B-cell development. It cannot be ignored however, that FNIP1 loss of function dramatically affects FLCN stability and activity, a consequence that must be taken into account. Our identification of FLCN as an Hsp90 client and FNIP1/2 as Hsp90 co-chaperones provides mechanistic insight into how FNIP1/2 stabilize FLCN and also suggests broader implications for these proteins in the cell.

Tsc1 and Tsc2: declaring independence

Tsc1 and Tsc2 were initially characterized as a protein complex that exerts inhibitory control on mTORC1 via the GAP activity of Tsc2. Tsc1 was later found to protect Tsc2 from HERC1-mediated ubiquitination and degradation, and we recently identified a mechanism for this; Tsc2 is a client of Hsp90 and Tsc1 is an Hsp90 co-chaperone, which helps load Tsc2 to the chaperone machinery. Much of the TSC literature treats the Tsc1-Tsc2 complex as a single entity and focuses on the activity of Tsc2 towards mTOR signaling, in spite of reports that Tsc1 and Tsc2 have unique tissue distributions and do not necessarily co-localize [5]. However, reports of separable functions of Tsc1 and mTOR-independent functions are relatively limited despite early knowledge that Tsc2-mutant TSC generally exhibits a more severe clinical phenotype than that resulting from *TSC1* mutations [6].

Tsc1 and FNIP1/2 as new Hsp90 regulators

As mentioned, we have established new *bona fide* functions for FNIP1/2 and Tsc1 as co-chaperones that regulate Hsp90 chaperone activity. FNIP1 and Tsc1 both exhibit complex, multi-domain binding to Hsp90 and both decelerate its ATPase activity, which is essential to Hsp90 function and progression through the various conformational states that make up the Hsp90 chaperone cycle. Both FNIP1 and Tsc1 also compete with the activating co-chaperone Aha1 for Hsp90 occupancy. The complement of other Hsp90 co-chaperones that

can be found in complex with FNIP1 and Tsc1 is overlapping, with some notable exceptions, which helps to provide clues to their distinct roles in this system.

Furthermore, FNIP1/2 and Tsc1 play roles in chaperoning not just the FLCN and Tsc2 tumor suppressors but a variety of other client proteins as well. Dramatic decreases in both kinase and non-kinase client proteins are observed in Tsc1 knockout cells, whereas overexpression of Tsc1 stabilizes non-kinase clients but destabilizes kinase clients [3]. Conversely, overexpression of FNIP1 or FNIP2 enhances and knockdown compromises Hsp90 kinase and non-kinase client protein stability [2].

Taken together, our observations suggest that these large co-chaperones participate in highly nuanced regulation of Hsp90. The recently elucidated roles for FNIP1/2 and Tsc1 as co-chaperones of Hsp90 provides a *bona fide* function for these proteins and sheds new light on their respective fields.

Concluding Remarks and Future Perspectives

Armed with the knowledge of these new functions for FNIP1/2 and Tsc1 as Hsp90 co-chaperones, re-examination of the literature may potentially yield new insights.

Much of the FNIP1 literature focuses on a role for FNIP1 as a negative regulator of AMPK activity. It should be noted, however, that multiple AMPK subunits are Hsp90 clients and the action of FNIP1 on AMPK is potentially mediated by Hsp90, though further exploration is warranted. Indeed several additional mTOR pathway components are dependent on Hsp90, including Akt, RAPTOR, and mTOR itself. Further, it is likely that other mTOR-dependent and metabolic-signaling-related effects attributed to FNIP1 function are in fact the result of modulation of FLCN function and stability via FNIP1 co-chaperone activity. This notion is supported by the observation that mTOR induction is mild in FNIP1^{-/-} mice as compared to mice lacking FLCN [7].

Tsc1 and Tsc2 effects are often conflated despite evidence demonstrating differences between them. The mTOR independent effects of Tsc1 loss reported in the literature may be related to the loss of its co-chaperone function and deregulation of clients other than Tsc2 [8–10]. Tsc1 function as a co-chaperone may also provide insight into deciphering the differences in phenotypic severity between TSC as a result of Tsc1 vs. Tsc2 loss [6]. We propose a model where Tsc1 co-chaperone loss leads to upregulation of mTOR signaling as a result of loss of Tsc2 stability, but that effect is dampened because, as stated earlier, activation of these mTOR-responsive pathways depends on Hsp90 chaperone function as well. This is in contrast to mTOR activation as a consequence of Tsc2 mutation, where Tsc1 co-chaperone activity, and thus Hsp90 chaperoning, is preserved.

Finally, we have also recently shown that there may be some ability for FNIP1 and Tsc1 to compensate for the loss of one another in their roles as Hsp90 co-chaperones in the chaperoning of tumor suppressors [11]. This finding supports the observation that there is synergistic hyperactivation of mTOR when both FNIP1 and Tsc1 are lost, as recently reported by Centini et al [12].

Taken together, mounting evidence suggests FNIP1/2 and Tsc1 regulation of the AMPK-mTOR axis is a product of their co-chaperone activity toward Hsp90. In order to fully appreciate the extent of the function of these proteins, however, we must focus not solely on the mTOR signaling pathway, but on the entire signaling network of the cell.

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Figure 1. Tsc1 and FNIPs are the new co-chaperone of Hsp90.

Hsp90 and its co-chaperones, FNIP1/2 and Tsc1 involvement in Tuberous Sclerosis Complex (TSC) and Birt-Hogg-Dubé (BHD) syndromes. Hsp90 client proteins are dark and light green, Hsp90 co-chaperones are orange, Hsp90 is red.