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JB Commentary

Arkadia—beyond the TGF-β pathway

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Arkadia, also known as ring finger 111 (Rnf111), is an E3 ubiquitin ligase that amplifies transforming growth factor (TGF)-β family signalling through degradation of negative TGF-β signal regulators, i.e. Smad7, c-Ski and SnoN. Arkadia plays critical roles in early embryonic development through modulation of nodal signalling, as well as progression of tissue fibrosis and cancer through regulation of TGF-β signalling. Recent findings suggest that, similar to other ubiquitin ligases, including Smurf1 and 2, Arkadia regulates signalling pathways other than those of the TGF-β family. Arkadia interacts with the clathrin-adaptor 2 (AP2) complex and regulates endocytosis of certain cell surface receptors, leading to modulation of epidermal growth factor (EGF) and possibly other signalling pathways.

Keywords: TGF-β/EGF/ubiquitin ligase/AP2 complex/endocytosis.

Abbreviations: AP2, clathrin-adaptor 2; EGF, epidermal growth factor; EMT, epithelial-mesenchymal transition; R-Smads, receptor-regulated Smads; TGF-β, transforming growth factor-β.

Arkadia is an E3 ubiquitin ligase, and was originally identified as a molecule that amplifies nodal signalling. Nodal is a member of the transforming growth factor-β (TGF-β) family, which is structurally similar to activin. Formation of the node, the equivalent of *Xenopus* Spemann's organizer in mammalian embryos, plays an essential role in specification of the axis during early embryonic development. Using gene-trap mutageneis, Episkopou and colleagues (*1*, *2*) discovered Arkadia as a molecule responsible for induction of the node through enhancement of nodal signalling.

TGF-β family signalling is transduced through Smad and non-Smad pathways (*3*, *4*). Arkadia is an intracellular protein containing a RING finger domain, and has been shown to enhance TGF-β family signalling through ubiquitin-dependent degradation of some intracellular proteins. Smad7, an inhibitory Smad, suppresses TGF-β family signalling through multiple mechanisms, including physical interaction with type I receptors for TGF-β family proteins, resulting in blockade of activation of receptor-regulated Smads (R-Smads; Smad2 and Smad3 for TGF-β, activin and nodal signalling) (*4*). Arkadia physically interacts with Smad7, induces ubiquitin-dependent degradation of it, and thereby enhances TGF-β family signalling (*5*). In addition to Smad7, Arkadia has been reported to induce degradation of phospho-Smad2/3, which may lead to efficient and maximal nodal signalling for rapid resetting of target gene promoters (*6*).

In addition, TGF-β family signalling is suppressed by the transcriptional co-repressor c-Ski and its related protein SnoN in the nucleus (*4*). c-Ski and SnoN have been reported to interfere with the interaction of R-Smads with transcriptional co-activators p300 and CBP, recruit histone deacetylases to Smad complexes, and disrupt the formation of Smad complexes, leading to attenuation of TGF-β family signalling. Arkadia binds to c-Ski and SnoN, and down-regulates the levels of their expression through ubiquitin-dependent degradation (*7*–*9*).

Since Arkadia enhances TGF-β family signalling, it plays pivotal roles in progression of various diseases in which TGF-β family signalling is involved. TGF-β induces tissue fibrosis, suggesting that Arkadia may be involved in the pathogenesis of some fibrotic disorders. Liu *et al*. reported that levels of expression of mRNAs for type 1 collagen, TGF-β1, TGF-β type I receptor, Smad7 and Arkadia were increased in a rat model of tubulointerstitial fibrosis, while that of Smad7 protein was decreased in the kidney. They suggested that Arkadia may play a major role in degradation of Smad7, induction of epithelial-mesenchymal transition (EMT) of tubular epithelial cells, and progression of tubulointerstitial fibrosis (*10*, *11*). In support of these findings, Gai *et al*. (*12*) reported that in *Trps1* haploinsufficiency mice (TRPS1 encodes a transcription factor and is responsible for tricho-rhino-pharyngeal syndrome), tubulointerstitial fibrosis was induced by increased phosphorylation of Smad3 and decreased expression of Smad7 protein. They also found that the level of expression of Arkadia was increased in the proximal tubule cells of $Trps1^{+/-}$ mice, and suggested that Arkadia played an essential role in the induction of EMT in these cells.

The function of Arkadia may also be linked to progression of cancer. Through induction of the degradation of c-Ski, Arkadia accelerates tumor metastasis of breast and lung cancer cells in mice, possibly by induction of EMT (*9*). A study using 20 human cancer cell lines revealed ubiquitous expression of Arkadia (*13*).

Levels of expression of c-Ski and SnoN proteins varied markedly and did not correlate with those of Arkadia protein in these cells. In some cancer cell lines, including diffuse-type gastric cancer OCUM-2MLN, Arkadia failed to degrade c-Ski protein, suggesting dysfunction of it in certain cancer cells. Since TGF-β signalling interferes with the progression of diffuse-type gastric cancer OCUM-2MLN *in vivo* (*14*), perturbations of the function of Arkadia may accelerate the progression of this type of cancer.

Smurf1 and 2 were originally identified as HECT type E3 ubiquitin ligases able to induce degradation of R-Smads and suppress TGF-β family signalling. Smurf1/2 also interact with inhibitory Smads (Smad6 and 7) and degrade type I receptors for the TGF-β family proteins. Smurfs thus exhibit biological activities opposite to those of Arkadia. However, it has been found that the targets of Smurfs are not restricted to the TGF-β family signalling proteins, and that they induce degradation of many other proteins, *e.g.* Runx2, RhoA, MEKK2, Axin and p53 (*15*, *16*).

Similar to Smurf proteins, Mizutani *et al.* (*17*) found that the function of Arkadia is not limited to regulation of TGF-β family signalling. Through yeast-two-hybrid screening, the μ2 subunit of clathrin-adaptor 2 (AP2) complex was identified as an Arkadia-interacting protein. The AP2 complex plays an essential role in the endocytotic machinery that links cargo membrane proteins to the clathrin lattice (*18*). The AP2 complex is composed of four subunits, *i.e.* α, β2, μ2 and σ2. The N-terminal portion of the μ2 subunit is known to be located at the centre of the AP2 complex, while the C-terminal domain of μ2 has been shown to physically interact with Arkadia (*17*). Arkadia induced ubiquitination of the μ2 subunit, and regulated endocytosis of epidermal growth factor (EGF) receptor induced by EGF (Fig. 1). Arkadia may thus regulate a wide variety of signalling processes, in which endocytosis of the cell surface receptors is regulated by the AP2 complex. Since Arkadia-null mice are embryonic lethal, analyses of conditional knockout mice lacking expression of Arkadia in certain tissues may disclose novel *in vivo* functions of Arkadia.

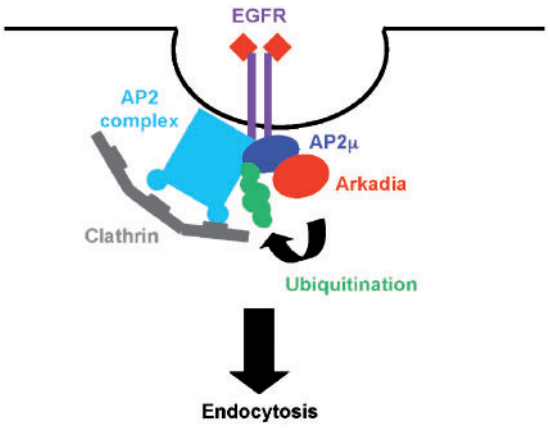

Fig. 1 Regulation of endocytosis of EGF receptor (EGFR) by Arkadia-AP2 complex. Currently, it is unknown whether AP2μ interacts with EGFR and Arkadia at the same time.

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