

# Putting the Rit in cellular resistance

## Rit, p38 MAPK and oxidative stress

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**C**ells mobilize diverse signaling pathways to protect against stress-mediated injury. Ras family GTPases play critical roles in this process, controlling the activation and integration of multiple regulatory cascades. p38 mitogen-activated protein kinase (MAPK) signaling serves as a critical fulcrum in this process, regulating networks that stimulate cellular apoptosis but also promote cell survival. However, this functional dichotomy is incompletely understood, particularly regulation of p38-dependent survival. Here, we discuss our recent evidence that the Rit GTPase associates with and is required for stress-mediated activation of a scaffolded p38-MK2-HSP27-Akt pro-survival signaling cascade. *Drosophila* lacking *D-Ric*, a Rit homolog, are susceptible to a variety of environmental stresses, while embryonic fibroblasts derived from Rit knockout mice display blunted stress-dependent signaling and decreased viability. Conversely, expression of constitutively active Rit triggers p38-Akt-dependent cell survival. Together, our studies establish Rit as the central regulator of an evolutionarily conserved, p38-dependent signaling cascade that functions as a critical survival mechanism in response to stress.

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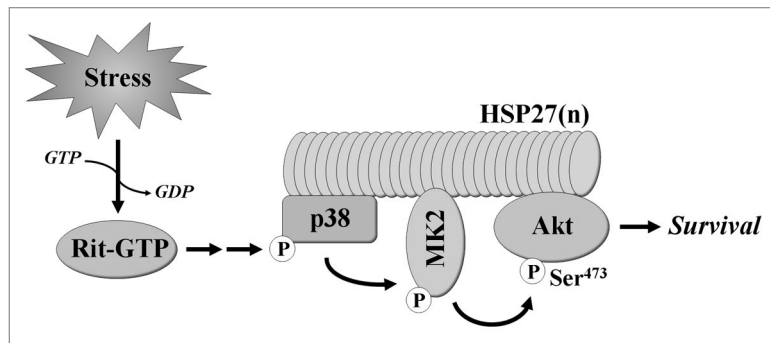
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differentiation, migration and survival.<sup>2,3</sup> The cellular balance between the extent and duration of the various MAPK cascades play a critical role in regulating cell fate, although the molecular mechanisms controlling whether stress-activated MAPK signaling, particularly p38 activation, results in cell death or recovery remain incompletely understood.<sup>2-4</sup>

Ras-related small GTP-binding proteins function as guanine nucleotide regulated molecular switches that govern physiological processes by regulating diverse effector pathways, including prominent roles in the control of MAPK signaling cascades.<sup>5</sup> While the physiological role for many Ras family GTPases is known, the cellular function for a number of “orphan” GTPases remain to be determined.<sup>5</sup> This includes Rit, the founding member of a novel branch of the Ras subfamily, sharing closest homology with Rin (Ras-like in neurons) and the *Drosophila* Ric (Ras-related protein that interacts with calmodulin) GTPases. The conservation of the Rit GTPase from flies to humans suggests conservation of an important physiological function(s). A series of studies using both expression of active and dominant-negative Rit mutants and RNAi-mediated gene silencing approaches had identified putative roles for Rit in the control of signaling pathways involved in neuronal morphogenesis,<sup>6,7</sup> differentiation<sup>8-10</sup> and cell survival.<sup>8,11</sup> However, whether any of these functions constitute an essential cellular role for Rit remained uncharacterized.

To critically assess the physiological function(s) of Rit, we begin by examining the impact of *D-Ric* GTPase loss in

The ability to resist injury following stress exposure is critical to cellular survival. The stress-activated JNK and p38 MAPK cascades have traditionally been viewed as promoting signaling networks which antagonize cell proliferation and survival.<sup>1</sup> However, it is now clear that JNK/p38 pathways function in a context-specific manner to modulate cell proliferation,



**Figure 1.** Rit signaling confers resistance to cellular stress. New evidence indicates that Rit plays a key role in stress-mediated cell survival by directing p38 MAPK toward the activation of MK2 and Akt within a HSP27 scaffolded signaling complex.

*Drosophila*. While vertebrates express 35 members of the Ras branch of the small GTPase superfamily, *Drosophila* express only 14 orthologs, including RIC, which shares an effector domain and > 65% amino acid identity with the vertebrate Rit and Rin GTPases.<sup>12,13</sup> *D-Ric* null flies were viable, fertile and displayed no obvious developmental defects. Because earlier studies had identified a role for Rit signaling in cell survival,<sup>8</sup> the susceptibility of *D-Ric* mutants to stress was examined. Adult flies displayed a reduced resistance to numerous environmental stresses, when compared with wild-type controls, indicating that RIC-dependent survival signaling cannot be compensated for by any other *Drosophila* GTPases. Moreover, the amplitude and duration of p38 MAPK signaling was blunted in null flies in response to heat shock, supporting a role for RIC-p38 signaling in recovery from thermal stress. To extend the analysis to vertebrates we generated a *Rit1* knockout mouse.<sup>14</sup> Rit null animals were born at the expected Mendelian ratio and displayed no gross morphological or anatomical abnormalities, suggesting that as seen with RIC deletion, Rit function is not essential for cellular proliferation or embryonic development.

To evaluate the contribution of Rit to cell survival, primary mouse embryonic fibroblasts (MEFs) were cultured from wild-type and Rit null littermates. Surprisingly, Rit<sup>-/-</sup> MEFs displayed a selective vulnerability to reactive oxygen species (ROS). Analysis of cellular kinase cascades found that hydrogen peroxide treatment resulted in the activation of p38,

ERK and ERK5 in wild-type MEFs. In contrast, the activation of p38, and ERK were reduced in Rit<sup>-/-</sup> MEFs, suggesting that Rit-dependent regulation of one or more of these pathways might contribute to survival in response to oxidative injury. Inhibition of p38, but not ERK signaling, was shown to disrupt Rit-dependent survival signaling in both primary MEFs,<sup>14</sup> but also in a variety of cultured cell lines.<sup>11</sup> These results motivated studies to explore the requirement for Rit in stress-mediated p38 activation. Silencing of Rit (using either RNAi-mediated knockout or Rit<sup>-/-</sup> MEFs) dramatically suppressed p38 activation downstream of a variety of stresses, and in a number of mammalian cell lines, supporting a general role for Rit in coupling cellular stress to a p38-dependent survival signaling.

The identification of p38 as a key element in Rit-dependent survival signaling was unexpected as p38 activation is commonly associated with the induction of cell death.<sup>3</sup> Further analysis found that, in parallel with inhibition of stimulus-mediated p38 activation, Rit silencing inhibited stress-induced Akt activation, mirroring the results from ROS stimulated Rit<sup>-/-</sup> MEFs.<sup>14</sup> Recent work has identified a pro-survival cascade in which p38 promotes Akt activation within a novel HSP27 scaffolded complex.<sup>15-18</sup> Within the complex, p38 activates MAPK activated protein kinase 2 (MAPKAPK2 or MK2), resulting in the phosphorylation of HSP27 and Akt, leading to cellular responses including the inhibition of apoptosis (Fig. 1).<sup>19</sup> Expression of activated Rit in PC6 cells was shown to stimulate MK2 activity and

increase levels of phosphorylated HSP27 in a p38-dependent fashion, while silencing endogenous Rit inhibited stress-mediated MK2 and HSP27 phosphorylation. Consistent with a role for Rit in the regulation of this cascade, MK2, HSP27 and Akt phosphorylation were decreased in Rit<sup>-/-</sup> MEFs following ROS exposure, when compared with similarly treated wild-type MEFs. Akt signaling is known to regulate the balance between cell survival and apoptosis.<sup>20</sup> Phosphorylation of Bad of S136, a pro-apoptotic member of the Bcl-2 family, results in its sequestration in the cytosol, promoting cell survival.<sup>21,22</sup> Consistent with a role for Akt signaling in MEF survival following oxidative stress, in Rit null cells, Bad S136 phosphorylation was significantly reduced.

Selective association of Ras and Rho family GTPases with scaffolded MAPK cascades is one mechanism known to confer specificity to MAPK signaling.<sup>23</sup> Consistent with a unique role for Rit in channeling ROS-mediated p38 activation to the MK2/HSP27/Akt pathway, using either dominant-negative mutants or RNAi silencing approaches to disrupt MK2 or HSP27, had no effect on Akt activation downstream of the closely related H-Ras and Rap1A GTPases. Moreover, co-immunoprecipitation studies found that p38, MK2, HSP27 and Akt were capable of associating with Rit, either individually or indirectly as part of a larger complex, but with the exception of p38, these proteins failed to associate with H-Ras. Together, these data support a model in which Rit signaling regulates a conserved survival function that involves Rit association with and regulation of a p38-MK2-HSP27-Akt signaling complex (Fig. 1).

The conservation of the Rit-p38 cascade from *Drosophila* to man highlights its importance to cell survival and potentially to the regulation of additional p38-mediated biological processes.<sup>3</sup> These include the regulation of pro-survival transcriptional cascades,<sup>24-26</sup> control of cell cycle progression<sup>27</sup> and the lifespan.<sup>28</sup> In future it will be important to determine whether Rit might modulate these, or other p38-dependent processes. In addition, while p38 inhibitors are under development for the treatment of inflammatory

and autoimmune disease, cancer and cardiovascular disorders, toxicity of these agents has been an issue.<sup>29</sup> Given the chronic nature of these disorders, and the complex regulatory role played by p38 signaling, greater insight into molecular mechanisms governing p38-mediated survival signaling may aid in the development of new therapies which limit adverse clinical outcomes.

In conclusion, the finding that Rit functions as a critical regulator of an evolutionarily conserved, p38-dependent survival signaling cascade, by directing p38 activity toward the activation of Akt within a HSP27 scaffolded complex, represents an important step forward in our understanding of Rit function and provides insight into a molecular mechanism coupling p38 activity to cell survival.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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