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Visions & Reflections (Minireview)

Isoform-specific functions of Akt in cell motility

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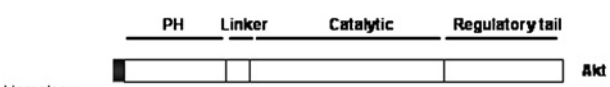
One of the stars of Dr. Dolittle's brigade of talking animals is the Pushme-Pullyu, a two-headed llama with a head at each end. Whenever one of its heads moves in its forward direction, the other is always backtracking, and vice versa. Recent studies on the Akt family of protein kinases (protein kinase B) have shown a similar duality in the function of its members in cell motility, with unique roles for isoforms in promoting migration.

The Akt family consists of three members, Akt1, Akt2 and Akt3, all of which are serine-threonine protein kinases activated through phosphoinositide 3-Kinase (PI3K)-mediated pathways. Akt signaling has been widely studied for its roles in cell survival and glucose metabolism [for a recent review see ref. [1]], but more recently has been implicated in cell motility. The development of tools to study specific isoforms of Akt has shown that distinct functions are predominantly associated with the different isoforms. Akt1 knockout mice are smaller than their wild-type counterparts, and cells from these mice undergo higher rates of apoptosis, suggesting an essential role of Akt1 in cell survival [2, 3]. Alternatively, Akt2 null mice develop type 2 diabetes and their cells have defects in glucose metabolism [4 – 6]. It is important to note that there is substantial overlap in the expression patterns of each isoform, indicating that it is the intrinsic properties of each, and not the tissue-specific expression levels, that underlie the difference in phenotypes. There is particularly high homology between the catalytic domain of Akt1 and Akt2, suggesting that their functional differences may not be dependent on their substrate specificity (Fig. 1).

The different properties of the isoforms may have therapeutic implications since Akt is over-expressed or activated in many human cancers, and drugs targeting Akt are under development as potential anti-neoplastic agents [7, 8]. The ideal drug would target both the anti-apoptotic and pro-migratory functions conferred upon cells by Akt signaling without promoting type 2 diabetes. The question that remains to be answered, however, is which isoform of Akt would be the best to inhibit?



Homology:	PH	Linker	Catalytic	Regulatory tail
Akt1/Akt2	80	46	90	66
Akt1/Akt3	84	40	88	76
Akt2/Akt3	76	17	87	70

Figure 1. Comparison of the Akt1 and Akt2 isoforms (% homology). The PH and linker domains distinguish isoforms in cell motility. Adapted from Kumar and Matison [8].

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Akt1 in cell motility

While it is well documented that Akt has a role in cell migration, it is still not clear what role each isoform plays. Akt1 and Akt2 have been studied extensively, but the role of Akt3 in cell motility is unknown. In fibroblasts, over-expression of Akt1 stimulates motility, while dominant negative Akt1 mutants inhibit motility [9, 10]. This trend is also seen in Schwann cells where, again, dominant negative Akt1 mutants reduce cell motility [11]. Endothelial cells and fibroblasts from Akt1 but not Akt2 knockout mice have reduced cell motility as well [12, 13]. This property may be widely conserved, because studies in the unicellular slime mold *Dictyostelium discoideum* have also shown that Akt1 orthologs regulate cellular motility, cell polarity and chemotaxis [14].

Several cell motility pathways have been identified that mediate the Akt cell motility signals. One Akt1-dependent pathway leads to the small GTPase Rac and its effector Pak, although the precise mechanisms of Rac and Pak activation have not been fully elucidated [9, 10, 15, 16]. In *Dictyostelium*, Akt activates Pak by phosphorylation [11]. Another key target may be nitric oxide synthase [12]. In conclusion, Akt1 is the predominant isoform promoting cell motility in fibroblasts, endothelial cells, Schwann cells and *Dictyostelium*.

Akt2 in cell motility

While Akt1 is the isoform that promotes cell motility in some cells, in other cells Akt2 directs cell motility. For example, in breast and ovarian cancer cell lines, over-expression of Akt2 but not Akt1 increases motility and invasion, subsequently making the cells more metastatic [17]. Localization studies using transfected breast cancer lines show that Akt2, but not Akt1, is localized in close proximity to the collagen matrix, suggesting that localization of the kinase, as opposed to its specificity, may be the primary determinant of its function in cell motility [17]. In other studies, down-regulating Akt1, but not Akt2, stimulated migration [18, 19]. Again, this would also suggest that Akt2 has stimulatory effects on cell migration, because Akt2 down-regulation suppresses migration while Akt1 down-regulation enhances it. In fact, over-expressing Akt1 actually blocked invasion [19].

Distinct downstream targets have been implicated in breast epithelial cells for the effects of Akt on cell motility. In one study, Akt1 over-expression caused degradation of NFAT, a transcription factor that promotes carcinoma invasion [19]. In another study, the ERK (MAPK) pathway was implicated [18]. Together, these studies show that, in breast epithelial cells, the primary isoform mediating cell motility is Akt2.

Opposing roles

While there is clear evidence that Akt1 stimulates migration in some cell types, and Akt2 stimulates motility in other cell types, remarkably, where tested, the two kinases appear to oppose each other's actions. For example, while Akt1 is generally agreed to be a positive regulator of Pak1, Akt2 inhibits Pak and Akt2 knockout fibroblasts have elevated levels of Rac and Pak. Akt2 knockout fibroblasts migrate faster than wild-type cells, while Ak1 knockout migrate more slowly [13]. Similarly, as discussed above, Akt2 expression stimulates breast cancer cell motility, while Akt1 expression inhibits cell motility. Therefore, a balance exists between the two kinases to regulate cell motility.

How can these two family members have distinct roles in cell motility? While the differences are not fully understood, a chimera-based approach has recently shed light on this question. To study the relationship between the Akt isoforms and cell migration, constructs between Akt1 and Akt2 were made in which functional domains were swapped. The different mutants were re-expressed in Akt2 knockout cells and tested. From these experiments, it was clear that the linker domain between the catalytic domain and the PH domain is the distinguishing factor between Akt2 and Akt1, while the kinase domains are interchangeable [13]. This is because constructs in which the Akt1 kinase domain was fused to the Akt2 gene rescued cells as well as the wild-type Akt2, while fusing other parts of Akt1 failed to rescue the Akt2 knockout cells. Note that reintroduction of kinase-dead Akt2 failed to rescue migration, implying that the catalytic activity of Akt2 is important for its effects on cell migration [13]. The differences between the two kinases lie within the linker and PH domains, perhaps reflecting differences in mechanisms of activation or localization.

Cancer

The central role of Akt in preventing apoptosis and promoting cell motility and proliferation suggests that Akt inhibitors are an attractive therapeutic option for the treatment of cancer, with early clinical trials underway. Since knockout mice of Akt1, Akt2 and Akt3 are all viable, an *in vivo* approach using transgenic mice expressing either Neu or middle T antigen

in breast tissue was able to test the isoforms directly [20]. In both models, loss of Akt1 delayed cancer development, while loss of Akt2 actually accelerated tumor growth. Akt3 knockouts had no significant effects [20]. Furthermore, the breast cancer tumors generally expressed low levels of Akt2 and higher levels of Akt1, suggesting that Akt1 not only promotes tumor development but may also be required for growth [20]. Thus, despite increased motility in Akt1 knockout cells, other roles of Akt1 in cancer, such as its critical role in preventing apoptosis, may override its affects on cell motility. Perhaps the tumors that may respond best to Akt1 inhibition will be fibrosarcomas, because motility will be reduced while apoptosis will be stimulated.

A growing body of evidence finds Akt central for motility. However, which 'head' of the Pushme-Pullyu leads the way, Akt1 or Akt2, depends on the cell type.

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