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Perspective

The Par-1/MARK Family of Protein Kinases

From Polarity to Metabolism

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adiposity, AMPK, aPKC, LKB, metabolism, polarity, Par

ABBREVIATIONS

Par	partitioning defective
aPKC	atypical protein kinase C
AMPK	AMP-activated protein kinase
Glut4	glucose transporter 4

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ABSTRACT

The Par-1 protein kinases are conserved from yeast to man and belong to a subfamily of kinases that includes the energy sensor and metabolic regulator, AMPK. Par-1 is regulated by LKB1 and atypical PKC and has been shown in multiple organisms and cell types to be critical for regulation of cellular polarity. Recent studies using knockout mice have revealed several surprising physiological functions for Par-1b/MARK2/EMK1. Our recent study shows that Par-1b regulates metabolic rate, adiposity and insulin sensitivity. This is the first study to implicate these kinases in metabolic functions akin to those previously defined for AMPK. Conversely, another series of recent publications now implicate AMPK in regulation of polarity. Here we discuss the metabolic phenotype seen in Par-1b deficient mice and the synthesis of several findings that link Par-1 and AMPK to a degree that has not been previously appreciated.

INTRODUCTION

The prototypical Par-1 kinase was identified in a seminal study published in 1988 designed to identify regulators of early embryonic polarity in *C. elegans.*[1] A series of subsequent studies by numerous groups demonstrated that Par-1, a serine/threonine protein kinase, is one of several evolutionarily conserved proteins (Par-1, Par-3/ASIP, Par-4/LKB1, Par-5/14-3-3, Par-6 and atypical PKC/PKC-3) required for cellular polarity not only in worms but also in flies, frogs and mammals.[2-16] This body of work has been reviewed elsewhere.[17-20] Studies done in the context of cellular polarity demonstrated that Par-1 mediates at least some of its effects by phosphorylating Par-3 and the microtubule associated protein Tau.[21-25] Numerous other potential Par-1 substrates have been identified including Cdc25C, KSR, Pkp2, Class II HDAC, Dlg and Rab11-FIP.[26-32] Interestingly, phosphorylation of several Par-1 substrates leads to the generation of phospho-dependent 14-3-3 binding. Par-1 substrates that fall into this category include Par-3, Cdc25C, KSR, Pkp2 and Class II HDAC. Both LKB1 (also known as Par-4) and atypical PKCλ/ι (or PKC-3 in *C. elegans*) regulate Par-1. LKB1 phosphorylates Par-1 and the related AMP activated kinase AMPK on an activation loop Thr residue. This modification is required for Par-1 activity.[33,34] LKB1 serves as a master regulator of cellular polarity, at least in part, by activating the Par-1 kinases.[16] Atypical PKC (aPKC) phosphorylates Par-1 to regulate its localization and kinase activity.[35-37] TAO-1/MARKK and GSK-3β have also been implicated as upstream regulators of Par-1.[38,39]

METABOLIC FUNCTIONS OF Par-1b/MARK2/EMK

Physiological functions of the mammalian Par-1 kinases have been revealed using targeted gene knockout approaches in mice. The mammalian Par-1 family is comprised of four members that go by several names (Par-1a/MARK3/C-TAK1, Par-1b/MARK2/EMK, Par-1c/MARK1 and Par-1d). Four studies have been published using two independently derived mouse lines null for Par-1b/MARK2/EMK.[40-43] These studies implicate Par-1b in a diverse set of physiological processes, including fertility, immune system homeostasis, learning and memory and growth and metabolism.

Our most recent study identifies a role for Par-1b in the regulation of metabolism.[43] Par-1b null mice are growth retarded (~20% reduced body mass relative to wild-type) as early as E13.5 and this growth retardation continues throughout the lifetime of Par-1b null mice.[43] Our results and those of Bessone et al. (1999) showing decreased serum IGF-1 levels in Par-1b null mice, provide a reasonable explanation for the observed

pre- and post-natal growth retardation. Although a growth hormone deficiency might explain postnatal growth differences, there is no evidence for a role of growth hormone (GH) in mammalian embryonic growth, suggesting that Par-1b deficiency leads to GH-independent growth defects. Although accurate determination of GH production is technically difficult, neither our study nor that of Bessone et al. (1999) detected altered serum GH levels in Par-1b null mice.

Morphometric analyses of Par-1b null mice revealed that although most tissues are proportionately smaller, knockout mice accumulate disproportionate decreases in adipose tissue (representing ~14% body fat in null mice versus ~23% in wild-type mice at twelve weeks of age). This differential is slightly increased with age (18% body fat in null mice versus 35% fat in wild-type mice at one year of age, n = 10 females per genotype, p = 0.002) (unpublished data). In addition to having reduced adiposity, Par-1b null mice are resistant to weight gain when placed on a high fat diet and are at the same time hyperphagic, eating twice that of their wild-type littermates. A likely explanation for these observations is the finding that Par-1b null mice are hypermetabolic. Thus, resistance to weight gain due to high fat diet or increased caloric intake is due to increased metabolic rate. Although the molecular mechanism for these metabolic changes is not clear, the data indicates that loss of Par-1b either directly or indirectly increases mitochondrial function in adipose tissue. Intriguingly, these perturbations are also accompanied by insulin hypersensitivity and improved glucose tolerance—possibly the result of compensatory changes that arise due to a chronic hypermetabolic state. Analysis of the relative levels of glucose uptake in muscle and fat indicates that adipose tissue is most dramatically affected in the absence of Par-1b. White and brown fat of Par-1b null mice exhibit increased glucose uptake in both basal and insulin-stimulated states.

Another possible explanation for enhanced insulin sensitivity and glucose uptake in Par-1b null adipose tissue is that Par-1b regulates GLUT4-mediated glucose uptake. Par-3/ASIP studies in 3T3L1 cells indicate that this protein (a downstream target of Par-1) can inhibit insulin induced glucose uptake[44] aPKC, a negative regulator of Par-1, has been shown to regulate insulin triggered glucose uptake in multiple studies.[44,45] It is unclear at this point how Par-3/ASIP or aPKC act to regulate glucose uptake. It is conceivable that aPKC influences glucose uptake via regulation of Par-1. Par-1 has also been implicated in the regulation of vesicular trafficking via the exocyst, which in turn has been shown to play a critical role in the transport of insulin-responsive Glut4 vesicles.[46-48] Regulation of Rab11-mediated functions might provide a mechanistic explanation for this model.[32,49] Thus, Par-1 may be involved in GLUT4-mediated glucose uptake via a mechanism(s) that involves Par-3, aPKC and/or the exocyst.

An argument against a direct role for Par-1b in early signaling events that control Glut4-mediated glucose uptake is our finding that insulin receptor proximal signaling (IRS-1 and AKT phosphorylation, phosphatidylinositol 3-kinase recruitment) is slightly decreased in adipose tissue from Par-1b null mice. Furthermore, Zhou et al. (2004) reported that knockdown of Par-1a and/or Par-1b in 3T3L1 cells by siRNA-treatment, has no effect on basal- or insulin-stimulated glucose uptake.[50] If knockdown was sufficient to block the function of both Par-1a and Par-1b in the 3T3L1 system and if there is not compensation by the other two Par-1 family members, then this data provides an argument against a direct role for Par-1 in Glut4-mediated glucose uptake. Even if Par-1 regulates the activity of Glut4 downstream of AKT, this function would not completely explain our observation of hypermetabolism and reduced adiposity in the Par-1b null mice because adipose-specific overexpression of Glut4 alone leads to enhanced glucose uptake in combination with increased adiposity (not decreased adiposity as observed in Par-1b deficiency).[51-53] Future studies using tissue-specific deletion of Par-1b in adipose tissue will elucidate the contribution made by adipose tissue to these phenotypes.

There is also a significant body of literature describing a role for Par-1 in neuronal cell polarity.[31,54-56] Several interesting possibilities, including regulation of the hypothalamic-pituitary-adrenergic axis of the neuronal system by Par-1b, might explain the observed metabolic changes in Par-1b null mice.[57] Again, tissue-specific deletion of Par-1b in the nervous system should clarify how this compartment contributes to the observed phenotypes.

AMPK AND PAR-1: PARTNERS IN POLARITY AND METABOLISM?

Based on sequence similarities (50% identity across their kinase domains), AMPK and Par-1 are closely related members of a subfamily that also includes BRSK1/2, QIK and SIK.[58] Conservation of the kinase domains of these proteins suggests similar phosphorylation-site preferences. Alignment of several known substrates indicates that Par-1 and AMPK prefer to phoshorylate a serine residue when Leu/Ile/Met, Arg/Lys, and Leu are present in the -5, -3 and +4 positions, respectively (Fig. 1). In addition to sharing a common consensus phosphorylation motif, both AMPK and Par-1 family members are activated by LKB1.[34] Interestingly, recent studies indicate that Par-1b and AMPK have overlapping functions in vivo. As mentioned above, Par-1 has historically been associated with regulating polarity, while AMPK has been studied for many years in the context of energy sensing and metabolism. The metabolic

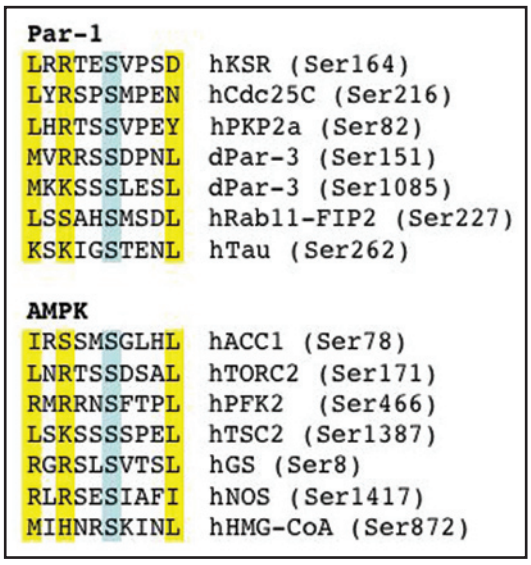

Figure 1. Par-1 and AMPK substrates share a common phosphorylation consensus sequence. Sequences inclusive of and surrounding the phosphorylation site of several Par-1 and AMPK substrates are aligned. Phosphorylated serine residues are highlighted in blue and preferred residues in the -5, -3 and +4 positions are highlighted in yellow. h, human; d, Drosophila; KSR, kinase suppressor of Ras; PKP2, Plakophilin, ACC1, acetyl CoA carboxylase; TORC2, transducer of regulated cAMP response element-binding protein (CREB); PFK2, phospho-fructo-kinase; TSC2, tuberous sclerosis; GS, glycogen synthase; NOS, nitric oxide synthase; HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A reductase.

functions of AMPK have been well-reviewed elsewhere.[59,60] These functions include inhibition of fatty acid, glycogen and protein synthesis and activation of glucose uptake (in skeletal muscle) and glycolysis in response to cellular energy stress (increased AMP levels). Our study now elucidates a role for Par-1b in metabolic regulation, potentially via an adipose- and/or a neuronal-specific mechanism(s). At the same time, four recent studies have uncovered a role for AMPK in the regulation of polarity.[61-64] It is therefore tempting to speculate that Par-1 and AMPK share either a common and/or complementary set of substrates that act to regulate similar biological processes.

CONCLUDING REMARKS

Recent studies using Par-1b null mice demonstrate that mammalian Par-1b/MARK2 is required for multiple physiological processes that could not have been predicted from previous studies conducted in vitro. Our recent work indicates that Par-1b is important for metabolic regulation, and in particular, adiposity. Perhaps not surprising, a combination of studies has now drawn several parallels between substrate specificities, upstream regulators and physiological functions of Par-1 and AMPK. Although their kinase domains are clearly related in sequence, the notion that the functions of Par-1 and AMPK in polarity and metabolism, respectively, might be overlapping, was not predicted. Many exciting questions remain to be answered, including the identification of downstream substrates and upstream regulators of Par-1 and AMPK with respect to their newly defined functions as well as the identification of tissues that are relevant to the observed metabolic defects in Par-1b null mice.

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