

PAQ receptors: a counteracting force to ceramides?

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Abstract

In recent years, sphingolipids have garnered increasing attention for their roles in modulating intracellular signaling events. Circulating factors associated with obesity promote excess accumulation of ceramide or glucosylceramide derivatives, which impair insulin action in peripheral tissues. In this issue, Villa et al. (2009) provide evidence that, in yeast, the PAQR superfamily of receptors mediate their effects via a novel ceramidase activity, generating sphingoid base as a second messenger.

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An epidemic rate of obesity and associated metabolic disorders may soon start to cause a decline in life expectancy, which has steadily increased for the past two centuries (Olshansky et al., 2005). Obesity increases the risk for numerous disorders, including insulin resistance, cardiovascular disease and diabetes. While it remains unclear exactly how obesity leads to insulin resistance (i.e. the inability to induce a normal anabolic response in peripheral tissues in response to physiological levels of insulin), excess accumulation of deleterious lipids and low levels of chronic inflammation have both been implicated as causal factors. Recent advancements in the ability to target disruptions in sphingolipid production have revealed potential roles for the sphingolipid ceramide in insulin resistance, β -cell failure, cardiomyopathy and vascular dysfunction [reviewed in (Holland and Summers, 2008)]. In stark contrast, the adipose-derived factor adiponectin (Acpr30), has emerged as an insulin sensitizing protein with protective effects on the β -cell, cardiomyocyte and vasculature [Reviewed in (Trujillo and Scherer, 2006)]. In the current issue of Molecular Pharmacology, Villa and colleagues provide evidence that suggests that yeast homologs of adiponectin receptors may function, in part, by modulating sphingolipid metabolism.

Adipocyte-specific secreted molecules, termed adipokines, have dispelled the notion of adipose tissue as an inert storage depot for lipids, and highlighted its role as an active endocrine organ that monitors and alters whole-body metabolism and maintains energy homeostasis. One of these adipokines that our lab first identified in the mid-90's (Scherer et al., 1995), adiponectin (also known as Acpr30, AdipoQ and GBP28), has gained significant attention recently as a mediator of insulin sensitivity. Three different forms of the protein have been investigated in a number of different laboratories. Full-length adiponectin circulates as a trimer, a hexamer and a higher order complex (HMW form). These three forms represent the complexes found under

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normal physiological conditions. A fourth form, globular adiponectin (gAd) is a simple trimer of head domains only, lacking the wildtype protein's collagenous domain, with potent pharmacological properties in muscle; however, the physiologic relevance of this form remains to be demonstrated (Yamauchi et al., 2002; Yamauchi et al., 2007).

Clinical studies have revealed low serum concentrations of adiponectin in patients with obesity, insulin resistance, and cardiovascular disease (Wang and Scherer, 2008). Studies from adiponectin knockout and transgenic mice have highlighted and confirmed its role in metabolism. Under basal conditions, mice heterozygous for the adiponectin locus display a 60% reduction in adiponectin serum levels and mild insulin resistance, while a more severe insulin resistance with glucose intolerance is observed in knockout animals and exacerbated by as little as two weeks of a high fat/high sucrose diet (Kubota et al., 2002; Maeda et al., 2002).

Adenoviral re-expression of full-length adiponectin largely restores plasma glucose and insulin levels, suggesting that the phenotype of these knockout animals is a direct effect of the lack of adiponectin in serum. Similarly, restoration of serum adiponectin partially restores insulin sensitivity in a lipodystrophic mouse model, and completely restores insulin sensitivity if leptin is also restored. Transgenic mice have also been developed which promote physiological (2-3 fold) overexpression of circulating full-length, wild-type adiponectin resulting in improved hepatic insulin sensitivity and postprandial lipid clearance in an FVB mouse strain (Combs et al., 2004). The elevated adiponectin secretion completely rescued the diabetic phenotype in leptin deficient (*ob/ob*) mice, preserving β -cell function and insulin sensitivity despite substantially increased fat mass (Kim et al., 2007)

The progestin and adipoQ receptors (PAQR) are a novel family of receptors which include adiponectin receptors 1 and 2 (AdipoR1 and AdipoR2), and the membrane progesterone

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receptors γ and β . AdipoR1, identified by its ability to bind the globular form of adiponectin, is ubiquitously distributed with higher expression in muscle (Yamauchi et al., 2003). AdipoR2 is expressed predominantly in liver and appears to display greater affinity for full length adiponectin [reviewed in (Kadowaki and Yamauchi, 2005)].

Adenoviral overexpression of AdipoR1 or 2, like the overexpression of the yeast homolog in the current paper, suggests that the receptors may offer some degree of constitutive activity (Yamauchi et al., 2007). When overexpressed in leptin receptor deficient mice, these receptors appear to improve insulin sensitivity and glucose homeostasis. Genetic ablation of adiponectin receptors has yielded conflicting results. Kadowaki and colleagues have demonstrated that ablation of either AdipoR1 or AdipoR2 promotes an insulin resistant phenotype. By contrast, Liu and coworkers determined that AdipoR2 deficient mice show superior glucose and lipid metabolism compared to wildtype controls, but are ultimately more susceptible to β -cell decompensation and diabetes despite their improved metabolism (Liu et al., 2007).

Mechanistically, adiponectin may stimulate AMP-activated protein kinase (AMPK) in muscle, liver, and β -cells. Yamauchi et al has provided the first strong evidence for the molecular mechanism of adiponectin action. Using isolated muscle or C2C12 myocytes, they demonstrated that treatment with the globular form of adiponectin leads to a rapid, transient phosphorylation of AMPK peaking at 5 minutes post-treatment (Yamauchi et al., 2002). AMPK phosphorylation leads to acetyl coA carboxylase inhibition (by 15 minutes), decreased malonyl CoA, and subsequent derepression of carnitine palmitoyltransferase 1 (CPT1) activity, causing increased fatty acid oxidation in muscle. Adenoviral overexpression of AdipoR1, but not AdipoR2, was sufficient to accentuate AMPK activation, perhaps suggesting distinct signaling

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mechanisms for the two receptors (Yamauchi et al., 2007). Rather, overexpression of AdipoR2 increased the expression of PPAR- α and its target genes in liver.

In yeast, overexpression of AdipoR1 or other PAQ receptors represses expression of the iron transporter FET3, providing a reliable reporter gene. Noting that the PAQR family of receptors contains significant sequence homology with alkaline ceramidase (an enzyme which cleaves ceramide to produce sphingosine and free fatty acids), Lyons and colleagues evaluated the yeast PAQ receptor Izh2p to determine if it functions via ceramidase activity. While it remains uncertain whether the enzyme itself possesses ceramidase activity, their data clearly demonstrate that, at least in yeast, this class of receptors mediates its effect via this enzymatic cleavage of phytoceramide to produce phytosphingosine (**Fig. 1**). To our knowledge this is the first report of a ligand activated receptor possessing this type of enzymatic activity.

Yeast made an ideal biological system to evaluate a sphingolipid-dependent mechanism, in part because yeast only possesses 2 ceramidase enzymes. By contrast, 5 human ceramidases have been characterized to date [reviewed in (Mao and Obeid, 2008)]. Distinguished by their pH optima, alkaline (3 isoforms), acid, and neutral ceramidase are respectively localized to the ER/Golgi, plasma membrane, and lysosome. To date, few studies have analyzed the influence of these enzymes on glucose metabolism; however, in cell culture models, their overexpression protects against lipid-induced insulin resistance in cultured myotubes (Chavez et al., 2005), and cytokine-induced β -cell apoptosis (Zhu et al., 2008).

Lipopolysaccharide, the proinflammatory component of gram negative bacteria, and the proinflammatory cytokines interleukin 1 β (IL-1 β) and tumor necrosis factor α (TNF α) all induce ceramide accumulation in a variety of tissues and cell types. Notably, the death domain of the TNF α receptor contains sphingomyelinase activity which, upon its activation, regenerates

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ceramide from sphingomyelin via the cleavage of choline (Peraldi et al., 1996). IL-1 has similarly been shown to promote the hydrolysis of sphingomyelin (Santana et al., 1996). The generation of ceramide and glucosylceramide are critical for TNF α -mediated insulin resistance (Grigsby and Dobrowsky, 2001; Tagami et al., 2002). This mechanism of action of the TNF receptors is an excellent example for a hormone-mediated signaling event that affects cellular glucose homeostasis via the modulation of sphingolipid metabolism.

Sphingolipid metabolism has garnered increasing attention for its roles in insulin resistance, beta cell failure, and cardiovascular disease. The development of new drugs and novel transgenic animal models have allowed for targeted disruption of sphingolipid metabolism. *In vivo*, ceramide and certain glucosylceramide derivatives have been shown to impair insulin action in peripheral tissues, promote lipotoxic apoptosis in pancreatic β -cells, and impair vascular and cardiac function.

Several factors have suggested a role for ceramide in the impairment of peripheral insulin actions. First, ceramide accumulates in insulin resistant rodents and humans. Second, circulating factors known to impair glucose homeostasis such as glucocorticoids, saturated free fatty acids, and TNF α are known to promote aberrant ceramide accumulation. Third, addition of short chain ceramide analogs to cultured muscle, liver, or adipose tissue is sufficient to impair insulin stimulated activation of Akt, a key regulator of insulin's anabolic effects. At least two distinct mechanisms link ceramide to the inhibition of Akt, as ceramide activates protein kinase C-zeta and protein phosphatase 2A to prevent Akt phosphorylation and promote its dephosphorylation, respectively. Important to the context of the current paper, ceramide does not impair insulin signaling upstream of Akt, and does not prevent sphingosine-induced activation of PDK1, an important kinase in Akt activation (Stratford et al., 2004).

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Noting these effects, the Summers lab recently evaluated the role of excess ceramide accumulation in the pathogenesis of insulin resistance and type 2 diabetes (Holland et al., 2007). Using pharmacologic inhibitors or mice with genetically impaired ceramide synthesis *in vivo*, glucocorticoid, saturated fat, or obesity-induced insulin resistance was inhibited. Moreover inhibiting ceramide synthesis prevented diabetes in the ZDF rat, consistent with previous studies implicating ceramide as a key mediator of lipotoxic apoptosis in the β -cell. Using similar approaches, independent groups have prevented or reversed atherosclerotic lesion formation in ApoE deficient mice (Hojjati et al., 2005; Park et al., 2004). Most recently, the Goldberg group demonstrated that ceramide is essential for cardiac dysfunction in a lipotoxic model of heart disease (Park et al., 2008).

Indeed aberrant accumulation of ceramide produces numerous metabolic insults, while the potent adipokine adiponectin protects against them. The potential connection of the two fields prompts a number of interesting and important questions and validation of these results in mammalian cell culture models and *in vivo* will provide exciting insights into the mechanisms of obesity and associated co-morbidities.

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Figure Legend:

Figure One. PAQR signaling is mediated by sphingolipid metabolism. In yeast, Izh2p leads to repression of the iron transporter FET3. This process is sequentially mediated by: 1)PAQ receptors binding to their cognate ligands, 2) the cleavage of phytoceramide to regenerate phytosphingosine and fatty acid, 3) activation of Pkh1/2p by sphingoid base, 4) activation of protein kinase A (PKA) by Pkh1/2p, 5) PKA-induced inhibition of the transcriptional co-activator MSN2/4p and nuclear export of AMP kinase (preventing inhibition of the transcriptional co-repressor NRG1/2p). In mammalian cells, circulating factors impair insulin sensitivity via aberrant ceramide accumulation.

