Review

MOLECULAR METABOLISM

Adiponectin, driver or passenger on the road to insulin sensitivity?

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ABSTRACT

Almost 20 years have passed since the first laboratory evidence emerged that an abundant message encoding a protein with homology to the C1q superfamily is highly specifically expressed in adipocytes. At this stage, we refer to this protein as adiponectin. Despite more than 10,000 reports in the literature since its initial description, we seem to have written only the first chapter in the textbook on adiponectin physiology. With every new aspect we learn about adiponectin, a host of new questions arise with respect to the underlying molecular mechanisms.

Here, we aim to summarize recent findings in the field and bring the rodent studies that suggest a causal relationship between adiponectin levels in plasma and systemic insulin sensitivity in perspective with the currently available data on the clinical side.

Keywords Mouse; Human; Ceramide; AMPK; Oligomeric complexes; AdipoR; Insulin sensitivity

1. INTRODUCTION

Adiponectin was first discovered and characterized as an adipokine with a number of different oligomeric forms in 1995 [1]. Intrigued by its prominent mRNA expression in differentiated adipocytes, two additional groups reported the identification of the same gene in the next year [2,3]. Its presence in plasma facilitated the purification and further characterization of the adiponectin protein [4]. Adiponectin peptide sequences are highly conserved among mammals [5]. The crystal structure of the homotrimeric globular adiponectin form revealed its structural resemblance to the complement C1q superfamily. This was expected due to high similarity at the primary amino acid level. Less expected was the striking structural similarity to the tumor necrosis factor (TNF) superfamily of proteins [6]. As such, adiponectin was the first example of a C1q family member whose structure was solved, on the basis of which a structural resemblance between the C1q and TNF superfamilies was established. However, despite the close structural relationship, there are no reports of adiponectin-mediated activation of TNF receptor family members.

Ever since its initial discovery, adiponectin has inspired widespread interest. Readily detectable in blood, stable upon collection and relatively inert to the method of collection and diurnal changes, its levels inversely correlate with multiple metabolic disorders and related diseases. Adiponectin can therefore serve as a potent clinical biomarker in humans and rodents. From the 10,000 studies over the past two decades since its discovery, it is widely appreciated that adiponectin exerts pleiotropic metabolic effects. Adiponectin sensitizes peripheral tissues to insulin and protects against inflammation and apoptosis. As for the molecular and cellular mechanisms, these effects are primarily exerted through the two adiponectin receptors 1 and 2, as well as the non-signaling binding protein T-cadherin. Adiponectin receptors (adipoR)-1 and -2 have been recognized as the transmembrane signal transducers for adiponectin ligands, with downstream effectors including sphingolipid targets, AMPK, PPARα, PPARγ and additional components. Many aspects of adiponectin signaling have recently been covered comprehensively in [7].

Although primarily produced by the adipocyte, plasma adiponectin is paradoxically reduced in obese subjects, in both sexes. This was first reported in Japan [8], and later confirmed in hundreds of papers, including multi-ethnic US populations [9]. This is a reflection of obesity-induced adipose dysfunction. The underlying reasons for the down-regulation are complex and frequently relate to an "unhealthy" fat expansion with ensuing hypoxia that leads to fibrosis and endoplasmic reticulum (ER) stress in adipocytes [10]. Mechanisms on obesity-induced adiponectin suppression will be discussed in further details below. By contrast, a subset of chronic disease states (chronic kidney disease, heart failure and pulmonary disease) prompt circulating adiponectin levels to increase, as it does in the cases of type 1 diabetes [11,12] and antibody-induced type B insulin resistance [13]. In these cases, adipose tissue increases adiponectin production, potentially to compensate for impaired insulin signaling. Administration of recombinant adiponectin in mouse models inhibits hepatic glucose production [14,15] and may promote fatty acid consumption in muscle [16] and these findings in rodents are generally consistent with the correlations seen in many clinical studies.

Of particular importance in the context of assessing whether the well-established causal relationships of adiponectin with insulin sensitivity in rodents translate into a meaningful cause and effect relationship in humans is a recent study by Gao and colleagues [17]. These authors

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performed a Mendelian Randomization Study, further commented on in [18], in which genetic adiponectin variants associated with varying plasma adiponectin levels were linked to insulin sensitivity measurements determined by euglycemic insulin clamp studies. The authors conclude that genetically determined adiponectin levels critically and positively associate with insulin sensitivity. Furthermore, they also conclude that this causal relationship between increased adiponectin and insulin sensitivity could be at least in part mediated through a reduction in adipose tissue mass. While this study certainly supports adiponectin levels as a driving force for insulin sensitivity in a clinical setting as well, it relies on the measurements of plasma adiponectin only and does not take into consideration a potential beneficial role for adiponectin within the adipocyte proper.

2. REGULATION OF PRODUCTION

Differentially-ordered adiponectin multimers (3, 6, and 18–36 subunits) exist stably in plasma, with little or no inter-conversion between the forms [19]. To this date, there is still no credible evidence for the existence of a processed form of adiponectin, resembling the globular form of adiponectin that lacks the collagenous tail, frequently used in *in vitro* studies. The complex structure with the associated multimerization of adiponectin critically relies on assembling factors, such as the protein folding enzymes and redox environment in the ER [20]. Trimer is the major form in human cerebrospinal fluid [21]. An interesting sexual dimorphism is observed in adiponectin multimer composition in mice and humans. High molecular weight (HMW, 18–36 multimers) adiponectin is generally more abundant in females, in both proportion and absolute amounts, while males lean more towards trimers or hexamers [22–24].

Only two adipocyte-derived factors display levels with an inverse correlation with fat mass, one is adipsin (complement factor D) the other one is adiponectin [25]. Adiponectin shows a marked reduction of its circulating levels and secretion rate in humans with obesity and insulin resistance [8,26]. More recent epidemiological studies suggested a positive association of plasma adiponectin level with the lower extremity fat mass, and a negative one with the truncal fat, correlations that were independent of ethnicity and gender [9]. Multiple transcription factors have been reported to drive adiponectin expression in obesity and type 2 diabetes. The adiponectin promoter contains binding elements for C/EBPα, PPARγ, SREBPs and LRH-1 (liver receptor homolog-1) [27]. Facilitated by SirT1, FoxO1 forms a complex with C/EBPα and activates adiponectin gene transcription. The complex formation is impaired when SirT1 is suppressed in dietary or genetic type 2 diabetes mouse models [28]. In contrast, FoxO1 binds to PPARγ and blocks its occupancy on the promoter of target genes, and this interaction is prevented by insulin signaling [29]. This mechanism applies to the transcriptional repression of adiponectin in the context of iron overload as well [30]. Other signaling pathways leading to transcriptional inactivation of adiponectin include the 5-hydroxytryptamine 2A receptor [31] and CREB–ATF3 [32]. While these transcriptional regulatory steps are unquestionably important, it is not clear whether they present rate-limiting control over adiponectin protein release from adipocytes or whether primary control on adiponectin levels are exerted at the level of post-translational handling of the protein in the secretory pathway.

This post-translational regulation of adiponectin involves its folding and processing in the ER, as well as its trafficking through the Golgi. ER chaperones ERp44 and Ero1α are crucial for assembly of high-order adiponectin complexes. Residue cysteine-39 is required for the covalent interaction with ERp44, and Ero1α mediates their dissociation [33]. DsbA-L was identified by yeast 2-hybrid and characterized as another key regulator of adiponectin multimerization [34]. There is also increasing evidence for specialized subcategories of secretory vesicles for different cargo molecules produced by the adipocyte. Adiponectin is packaged in vesicles distinct from leptin containing vesicles and is concentrated in "Golgi-localizing γ-adaptin ear homology ARF-binding protein" (GGA)-1 coated vesicles [35].

While the adipocyte is well established as the predominant cell type to produce adiponectin, emerging data suggest cardiomyocytes [36–39], skeletal muscle cells [40–46], and other tissues as alternative sources of adiponectin [47]. At this point, it is not clear whether the mRNA and protein pools described in these alternative tissues represent significant levels of expression that would be high enough to make a difference locally, or be sufficiently high to affect circulating pools of adiponectin. Furthermore, induction of adiponectin in these tissues may critically depend on the specific (patho-)physiological state or on a pharmacological intervention. Lastly, many of these cell types are in close physical proximity to adipocytes, and hence contamination of preparations with fat cells cannot be fully ruled out. A better appreciation of the relevance of local adiponectin expression levels will have to await cell type-specific functional inactivation of the adiponectin gene.

3. ADIPONECTIN RECEPTORS AND DOWNSTREAM SIGNALING

Adiponectin receptors (adipoRs)-1 and -2 were first cloned in 2003 [48]. Overexpression and siRNA knockdown of adipoR1 and R2 revealed their important roles in cellular binding of adiponectin, as well as the downstream AMPK and PPARα signaling. AdipoR1 mRNA is detectable in skeletal muscle, spleen, lung, heart, kidney, and liver. AdipoR2 is expressed in liver, but also detectable in heart, lung, skeletal muscle, and kidney [48].

Adenoviral overexpression of adipoR1 or R2 in the liver of *db/db* mice demonstrated distinct signal transduction and physiological outcomes. AdipoR1 modulates the activation of AMPK and suppresses expression of gluconeogenic and lipogenic genes, though the involvement of AMPK in this process in the liver has been questioned [49]. AdipoR2 induces PPARα, thereby increasing glucose uptake and fatty acid oxidation, as well as leading to a reduction of inflammation and oxidative stress [50]. Targeted disruptions of adipoR1 and R2 also support their roles in metabolism, though nuances exist, depending on which mice are examined. Studies utilizing the knockout mouse models generated by Deltagen (San Carlos, CA) showed that disruption of adipoR1 in males resulted in mild decreases in glucose intolerance, physical activity and energy expenditure, along with increased adiposity under regular chow conditions [51]. Surprisingly, adipoR2 knockout mice displayed resistance to high fat diet-induced obesity and dyslipidemia, along with increased glucose tolerance, insulin sensitivity, physical activity, and energy expenditure [51,52]. With inhibited β cell hyperplasia and hyperinsulinemia, the adipoR2 null mice became more hyperglycemic after a prolonged high fat diet regimen [52]. A mutual compensatory upregulation in the individual knock-outs can however not be ruled out, and double null mice have not yet been generated successfully with these Deltagen generated null strains. When Kadowaki and colleagues studied their own, independently generated null models of adipo R1 and R2, their findings were complimentary to their overexpression experiments, consistently pointing to the metabolically beneficial effects of

