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Wnt signalling at the crossroads of nutritional regulation

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Abstract

The ability to sense and respond to nutritional cues is among the most fundamental processes that support life in living organisms. At the cellular level, a number of biochemical mechanisms have been proposed to mediate cellular glucose sensing. These include ATP-sensitive potassium channels, AMP-activated protein kinase, activation of PKC (protein kinase C), and flux through the hexosamine pathway. Less well known is how cellularly heterogeneous organs couple nutrient availability to prioritization of cell autonomous functions and appropriate growth of the entire organ. Yet what is clear is that when such mechanisms fail or become inappropriately active they can lead to dire consequences such as diabetes, metabolic syndromes, cardiovascular diseases and cancer. In this issue of the *Biochemical Journal*, Anagnostou and Shepherd report the identification of an important link between cellular glucose sensing and the Wnt/ β -catenin signalling pathway in macrophages. Their data strongly indicate that the Wnt/ β -catenin pathway of Wnt signalling is responsive to physiological concentrations of nutrients but also suggests that this system could be inappropriately activated in the diabetic (hyperglycaemic) or other metabolically compromised pathological states. This opens the exciting possibility that organ-selective modulation of Wnt signalling may become an attractive therapeutic target to treat these diseases.

Keywords

glucose sensing; glycosylation; hexosamine pathway; metabolism; Wnt signalling

Coupling nutrient availability, prioritization of cellular functions and successful growth of a complex organ requires a co-ordinated response to a nutritional stimulus by all of its cellular components. The Wnt signalling network is a good candidate for co-ordinating these complex intra- and inter-cellular signals via autocrine and paracrine events. First, it is ideally suited to fulfil an integrative regulatory role as it is composed of a large network of highly regulated ligands, receptors, co-receptors and inhibitors all with potential to be differentially expressed in a cell-specific manner. In this way the Wnt signalling networks can provide an effective intercellular communication system that maintains the co-ordination of biological

functions and the entropy of a complex cellular system. Secondly, it is now becoming apparent that this signalling network is responsive to nutritional cues.

The name Wnt is derived from a combination of *Wg* (the *Drosophila wingless* gene) and *Int* [the murine homologue MMTV (mouse mammary tumour virus) integration site 1 gene]. It represents a large morphogenic ligand family comprised of 19 secreted lipid-modified glycoproteins that control multiple developmental processes during embryogenesis such as cell fate specification, progenitor cell proliferation and the control of asymmetric cell division. Wnts also regulate the maintenance and remodelling of adult tissues and organs, e.g. adult limb formation during metamorphosis, bone development, adipose tissue plasticity and cancer progression. Given that this family regulates many diverse processes, it is not surprising that the signal transduction pathways utilized are equally diverse. Currently, the Wnt signalling pathways can be categorized into two distinct groups: the Wnt/ β -catenin signalling pathway (formerly the canonical pathway) [1], and β -catenin-independent pathways (formerly the non-canonical pathways) which include the PCP (planar cell polarity) pathway, the Wnt/ Ca^{2+} signalling pathway and a number of others [2].

A central feature of the Wnt/ β -catenin signalling pathway is the regulation of cytosolic β -catenin protein levels through regulation of a destruction complex [comprised of CK1 α (cyclin-dependent kinase 1 α), GSK β (glycogen synthase kinase 3 β), APC (adenomatous polyposis coli) and Axin]. In the absence of Wnt signals, an active destruction complex creates a hyperphosphorylated β -catenin, which is targeted for ubiquitin-mediated degradation. Binding of Wnt ligands to a Frizzled/LRP (low-density lipoprotein receptor-related protein)-5/-6 receptor complex leads to the disassembly of the destruction complex and accumulation of cytosolic β -catenin. β -Catenin is then free to enter the nucleus and activate the transcription of Wnt/ β -catenin target genes through its interaction with LEF (lymphoid enhancer-binding factor)/TCF (T-cell-specific transcription factor) family of transcription factors. Wnt target genes include genes that affect cell proliferation (i.e. *c-myc*, *cyclin D1* and *Id2*) and differentiation and also the expression of proteins (i.e. Dkk1 and Axin2) that mediate negative feedback of Wnt/ β -catenin signalling.

Through these transcriptional actions, the Wnt/ β -catenin pathway plays a key role in controlling stem cell organization and maintenance, embryonic development, tissue differentiation, cell adhesion and migration, as well as tumorigenesis and cancer induction and progression. As shown by Anagnostou and Shepherd in this issue of the *Biochemical Journal* [3] and others, Wnt signalling also serves a pivotal role integrating cellular responses to metabolic and nutritional cues. As such the Wnt signalling network has the necessary flexibility and diversity to be well suited to co-ordinate cellular responses to specific nutritional perturbations. The relevance of this metabolic link has been illuminated by reports showing fundamental roles of Wnt signalling in the regulation of key metabolic organs such as muscle [4], liver [5], β -cells [6] and adipocyte determination, maintenance, proliferation, and differentiation [7,8].

In this issue of the *Biochemical Journal*, Anagnostou and Shepherd [3] present convincing evidence to demonstrate that physiological concentrations of glucose, one of the most available bioenergetic substrates, is able to stabilize β -catenin. They describe an elegant

series of pharmacological studies to dissect the mechanism, and identified that the Wnt/ β -catenin signalling pathway is the main effector pathway of the glucose-induced effects on cytosolic β -catenin in two macrophage cell lines.

According to this study, the mechanism involved in glucose-induced activation of the Wnt/ β -catenin signalling pathway seems to require activation of the HBP (hexosamine biosynthetic pathway), ultimately promoting N-linked glycosylation of Wnt-related proteins. The HBP is responsible for shuttling glucose to cellular glycosylation events. At the biochemical level, the enzyme GFAT (glutamine:fructose-6-phosphate amidotransferase) catalyses the conversion of fructose 6-phosphate (from glycolysis) into glucosamine 6-phosphate with glutamine acting as an amino donor. The final product of this pathway is the substrate UDP-GlcNAc (UDP-*N*-acetylglucosamine), which is the carbohydrate moiety used for O-linked glycosylation of serine or threonine residues on many intracellular proteins. This 'O-GlcNAcylation' of intracellular proteins is catalysed by the enzyme OGT (O-linked *N*-acetylglucosamine transferase) and can compete with phosphorylation sites. This aspect has been implicated in controlling proteasome function, gene transcription and neurofilament assembly, but also in diabetes and neurodegenerative disorders. UDP-GlcNAc can also be used in the Golgi N-glycan-branching pathway to mediate N-linked glycosylation of cell-surface receptors, transporters and secreted proteins. The first step of this process is catalysed by the enzyme GPT (GlcNAc phosphotransferase), which is located in the Golgi and transfers *N*-acetylglucosamine-1-phosphate from UDP-GlcNAc to dolichyl phosphates in newly synthesized proteins. Since HBP is dependent on the substrates that are key metabolites in carbon, nitrogen, and energy homeostasis (fructose 6-phosphate, glutamine, acetyl-CoA and UTP) it is thought to provide a cellular mechanism for sensing changes in metabolic flux and also links these to appropriate responses, such as cellular transition between growth and arrest.

Anagnostou and Shepherd [3] demonstrated the involvement of the hexosamine pathway in glucose-induced β -catenin accumulation by targeting GFAT (the rate-limiting enzyme in the HBP). First, they show that, similar to GFAT activity, glucose-induced β -catenin accumulation required glutamine. Furthermore, blocking GFAT activity with known inhibitors, azaserine and DON (6-diazo-5-oxonorleucine), also prevented glucose-induced β -catenin stabilization in macrophages. To further implicate the involvement of N-linked glycosylation, Anagnostou and Shepherd [3] demonstrate that tunicamycin pre-treatment inhibited glucose-induced β -catenin stabilization. Tunicamycin is a mixture of homologous nucleoside antibiotics that inhibit GPT and block the synthesis of all N-linked glycoproteins. Of note is the fact that this inhibition leads to the accumulation of unfolded or misfolded proteins in the lumen of the endoplasmic reticulum, which has been invaluable to its use as a tool to induce the cellular UPR (unfolded protein response).

Anagnostou and Shepherd [3] also showed that the glucose-induced increase in β -catenin protein is the result of inhibition of its degradation and that this also coincided with induction of β -catenin/TCF target genes such as *Axin2* and *cyclin D1*. Although there are a number of possible mechanisms that can alter β -catenin stability, Anagnostou and Shepherd [3] show that glucose-induced β -catenin stabilization requires the functional activity of extracellular Wnt ligands, because the effect of glucose was blocked by the extracellular

Wnt antagonists Dkk1 (dickkopf 1) and sFRP2 (secreted frizzled-related protein 2). Although the mechanism appears to be independent of alterations in GSK phosphorylation, it did correlate with the activation and phosphorylation status of the Wnt co-receptor LRP-6.

Therefore the results presented by Anagnostou and Shepherd [3] clearly point to the engagement of the Wnt/ β -catenin pathway in glucose-induced accumulation of β -catenin. However, in our opinion these results do not preclude the possibility that glucose or other nutrients may also exert metabolic effects through β -catenin-independent Wnt signalling pathways. Thus the metabolic effects of β -catenin-independent Wnt pathway can still be considered 'comanche' territory requiring further exploration. Similarly, there is evidence that the Wnt tone can be regulated by specific extracellular secreted antagonists, such as sFRPs, WIF (Wnt-inhibitory factor) and Cerberus, and intracellular modulators such as sclerostin or Dappers, which altogether contribute to ensure a balanced titrated response to specific nutritional external stimulus. The effect of nutritional cues on these Wnt signalling modulators should now be the subject of future research.

As is common with most significant findings that provide a spotlight on a new area of biology, they also raise more questions that need to be answered. For example: does this effect of glucose on β -catenin alter macrophage function? Does this effect on β -catenin occur in macrophages *in vivo*? Does it occur in other cell types or is it restricted to those that are specialized for glucose sensing? Are these mechanisms involved in or deregulated in disease states?

Since the work of Anagnostou and Shepherd [3] was performed in macrophage cell lines, and although these cells were selected for the convenience of their lack of plasma membrane pool of β -catenin, the observed glucose-dependent effects are likely to be directly relevant to macrophage function and proliferation. Indeed, this study lends further support to the roles of nutritional cues and Wnt signalling in immune function [9,10]. However, it remains to be seen whether glucose-dependent effects on macrophage Wnt/ β -catenin signalling are observed in all macrophage populations, including those resident in metabolically relevant tissues (i.e. liver, brain and adipose tissue). Such investigations could have significant implications on our understanding of certain leukaemias as well as pro-inflammatory diseases such as inflammatory bowel disease, rheumatoid arthritis, obesity and atherosclerosis.

Globally considered, the observations by Anagnostou and Shepherd [3] together with existing evidence linking nutritional status to specific changes in Wnt signalling tone, also reinforces the concept that the Wnt/ β -catenin signalling pathway is fundamental for the adaptation of organs to specific bioenergetic demands and changing nutritional states. One of these organs where Wnt/ β -catenin signalling is fundamental for its development and functionality is adipose tissue. Following the seminal evidence by Ross et al. [11] to show that Wnt/ β -catenin signalling exerts inhibitory effects on the programme of adipocyte differentiation, accumulating evidence indicates that inactivation of Wnt/ β -catenin signalling is a physiological regulatory step required for the pro-adipogenic response to nutrients. However, our understanding of the molecular mechanisms is still in its infancy, since it is clear that regulation of Wnt signalling networks is likely to be far more complex. Indeed,

Kozak and co-workers have elegantly highlighted the fact that Wnt signalling is also regulated by nutritionally induced epigenetic re-programming and that this effect is relevant for adipose tissue expansion [12].

Whether deregulated Wnt/ β -catenin signalling also plays an important pathophysiological role in obesity-associated metabolic syndrome is currently an area of much interest. Support for this notion comes from work from our laboratory and others to suggest that signals induced by anti-adipogenic cytokines, such as TNF- α (tumour necrosis factor- α), can also stabilize β -catenin and activate Wnt/ β -catenin target genes [13]. Given that in the context of obesity and insulin resistance adipose tissue is infiltrated by macrophages that secrete these anti-adipogenic pro-inflammatory cytokines, it is tempting to speculate that these pathological signals may be involved in deregulating the normal nutritional regulation of Wnts, thereby inappropriately limiting further adipose tissue expansion [14,15].

Another significant aspect highlighted by the study of Anagnostou and Shepherd [3] is that the effect of glucose-induced stabilization of β -catenin is physiologically important because it indicates a direct effect of glucose on activating Wnt/ β -catenin signalling. Interestingly, this effect may be exclusively dependent on glycaemia, since it seems to be observed in the liver of insulin-deficient hyperglycaemic streptozotocin-treated rodents. It is, however, unclear whether this effect occurs in other tissues whose uptake of glucose transport is dependent on appropriate insulin action. If so, are these pathways deregulated under conditions of insulin resistance and hyperglycaemia (Type 2 diabetes)? In our opinion it would be important to clarify whether these effects of glucose on Wnt signalling are different in insulin-responsive and non-responsive tissues or cell types. This is important since elevated glucose in the context of obesity and insulin resistance may on the one hand enhance Wnt signalling in adipose-tissue-resident macrophages while removing the responsiveness of these pathways in pre-adipocytes or adipocytes. Obviously, the concept of cellular-dependent Wnt signal activation in response to glycaemia levels is difficult to interpret in the context of whole tissue/organ characterization of Wnt signalling, unless the specific cells are isolated and analysed independently. However, the global interpretation of the events in a specific organ requires an integrated view of these effects, since it is likely that engagement of Wnt signalling networks also have paracrine effects on other cells. In short, although it is clear that Wnt signalling is responsive to nutritional perturbations, the pathophysiological relevance of these observations can only be truly appreciated by identifying the specific response of the cells integrated in that tissue, ideally using *in vivo* experimental settings. Only by this approach would it be possible to identify the co-ordinated cellular mechanisms that ensure positive but also negative responses to facilitate titrated bioenergetically efficient proliferative/differentiation cellular responses aiming to maintain the entropy of the organ under conditions of excess or scarce nutrient supply.

The implications of the nutritional control of Wnt signalling go beyond the purely metabolic angle. In fact, as Anagnostou and Shepherd [3] suggest in their manuscript that the link between glucose and Wnt signalling may be relevant to understand the behaviour of cancer, and particularly its increasing association with obesity and diabetes. Although there is clear evidence that cancer development requires escaping cell cycle control and that dysregulation of Wnt signalling is involved in its pathogenesis, few studies have addressed the

bioenergetic and biosynthetic requirements associated with uncontrolled growth. In fact targeting cancer at the level of lipid biosynthesis and or mitochondrial function are emerging as global strategies to treat cancer. In this respect, uncoupling Wnt responses from specific nutritional factors may provide an additional therapeutic angle if not to cure, then at least to decrease growth, delay relapse and/or prevent metastatic disease.

In summary, Anagnostou and Shepherd [3] provide evidence that glucose can exert direct effects on the Wnt/ β -catenin pathway, thereby providing a link between nutrients and the same signalling networks that are capable of orchestrating organ-specific responses to ensure co-ordinated metabolic responses and growth. This observation, together with other available evidence, identifies Wnt/ β -catenin signalling as a promising therapeutic area not only for obesity, diabetes and associated metabolic diseases, but also other areas such as atherosclerosis, rheumatoid arthritis and cancer.

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