Nuclear FoxO1 inflames insulin resistance

John E Dominy^{1,2} and Pere Puigserver^{1,2,*}

¹Department of Cancer Biology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, USA and ²Department of Cell Biology, Harvard Medical School, Boston, MA, USA

*Correspondence to: pere_puigserver@dfci.harvard.edu

The EMBO Journal (2010) 29, 4068–4069. doi:10.1038/emboj.2010.305

Chronic activation of macrophage-mediated inflammatory signals in insulin-sensitive metabolic tissues is thought to be one of the causes of insulin resistance—one of the hallmarks of the metabolic syndrome. However, the mechanisms, components and dynamics by which this inflammatory response is spatially and temporally regulated are not completely understood. In this issue of *The EMBO Journal*, Olefsky and colleagues (Fan *et al*, 2010) report that FoxO1, which is suppressed by the action of AKT, triggers the Tlr4 inflammatory signalling pathway in macrophages. The authors further establish that inflammatory signalling pathways are also able to signal back to and suppress the activity of FoxO1 through AKT. The findings of this paper suggest an intriguing regulatory transcriptional/signalling loop in macrophages that may contribute to maintain and exacerbate inflammation and insulin resistance in other cell types such as adipocytes.

Studies by many different laboratories have provided key evidence that, in both mouse models and humans, chronic inflammation is one of the pivotal mechanisms by which obesity is linked to insulin resistance (Hotamisligil *et al*,

1993; Weisberg *et al*, 2003; Xu *et al*, 2003; Cai *et al*, 2005; Solinas *et al*, 2007). Collectively, the picture that emerges from this research is that there is a hand-in-hand relationship between the signal transduction pathways that are involved in the control of the inflammatory cascade in insulin-sensitive metabolic tissues and the development of insulin resistance. The work from Olefsky's laboratory published in this issue of *The EMBO Journal* adds another important piece to this picture from the vantage point of macrophage-mediated inflammation and the Forkhead transcription factor, FoxO1 (Figure 1).

FoxO proteins are direct substrates of Akt, which controls FoxO transcriptional activity by regulating its cytoplasmic/nuclear translocation (Brunet *et al*, 1999). Due to the pivotal role of Akt in a diverse array of biological processes, the activity of FoxO proteins has been implicated in apoptosis, cell cycle, inflammation and metabolic pathways. Interestingly, FoxO1 is thought to account for a large part of the transcriptional metabolic effects of insulin signalling in mammals (Accili and Arden, 2004). For example, part of the suppressive effects of insulin on hepatic glucose output is

Figure 1 FoxO1 activates Tlr4 inflammatory pathway in macrophages and affects insulin resistance. Nuclear FoxO1 induces expression of genes encoding for proteins in the Tlr4 signalling pathway sensitizing macrophages to LPS (infection) and free fatty acids (diet). Once activated, Tlr4 signalling increases gene expression and secretion of pro-inflammatory cytokines, which promote insulin resistance in cell types such as adipocytes.

mediated through FoxO1 inhibition (Puigserver *et al*, 2003). In adipose tissue, FoxO1 activation suppresses adipocyte differentiation through direct repression of PPAR γ (Dowell *et al*, 2003). Here, Fan *et al* report that a key function of FoxO1 in macrophages is to activate the pro-inflammatory Tlr4 pathway. Moreover, their studies also implicate FoxO1 as a mediator of the known suppressor effects of insulin on inflammation.

Using ChIP-Seq analysis on serum-starved cells, Fan *et al* mapped regions of the chromatin that were bound by active FoxO1. Among the myriad genes that were identified as being occupied by FoxO1, the researchers observed a significant enrichment of genes involved in the Toll receptor-like signalling cascade. Tlr4 in particular was one of the most highly occupied genes within this subset of FoxO1 targets. The authors were able to establish functional transactivation of Tlr4 by FoxO1 through a series of reporter assays. Furthermore, they were able to demonstrate that activation of FoxO1, either using culturing conditions of minimal insulin signalling or genetically by overexpression of non-AKT phosphorylatable mutants of FoxO1, significantly potentiated Tlr4 signalling in part by increasing the expression of the Tlr4 receptor. Interestingly, the authors also observed that Tlr4 activation in macrophages resulted in the activation of Akt that, in turn, led to FoxO1 phosphorylation and its translocation to the cytoplasm, thereby terminating its transcriptional activity on the Tlr4 pathway. This negative feedback loop allows Tlr4 to attenuate its own inflammatory response in macrophagic cells.

The Tlr4 receptor can be activated by exogenous pathogenic ligands, such as LPS, or by endogenous ligands, such as saturated fatty acids (Fessler *et al*, 2009). The biology of this receptor could thus provide a causal link between the chronic consumption of high-fat/carbohydrate diets and the activation of pro-inflammatory processes *in vivo*. Indeed, the authors were able to show that FoxO1 enhances fatty acid-mediated increases in Tlr4-mediated signalling in RAW264.7 cells acutely cultured with saturated free fatty acids.

Consistent with chronic activation of Tlr4, the authors also provided evidence in high-fat-diet obese/insulin-resistant mice that FoxO1 is cytoplasmically sequestered in macrophages. Nevertheless, it is not yet clear in this experimental model whether Tlr4 signalling is indeed sustained in response to high-fat diet and to what extent FoxO1 is necessary for the maintenance of chronic inflammation under conditions of insulin resistance. Fan *et al* do show, however, that whole-body haploinsufficiency of FoxO1 does afford some measure of protection against lipopolysaccharide-induced inflammation and insulin resistance. This tantalizing piece of evidence suggests that additional investigations are warranted to tease out the signalling tryst between FoxO1, chronic inflammation and insulin resistance.

Overall, one of the most significant contributions of the work of Fan *et al* is their precise identification of the chromatin regions occupied by FoxO1, in particular elements that control expression of genes of the Tlr4 pathway (as well as many other gene pathways that have not yet been further characterized). This study raises many intriguing questions about the causal role of FoxO1 in inflammation and the development of insulin resistance. It also raises important questions about the effects of diet on sculpting the inflammatory response. Under physiological conditions of minimal PI3 kinase/AKT signalling, such as nutrient restriction or starvation, are inflammatory genes induced in macrophages as a consequence of Foxo1 activation? Whatever the answers to these questions turn out to be, the precise genomic mapping of FoxO1 targets offered by this paper, with particular regard to the Tlr4 signalling components, provides a unique starting point to determine additional key components of this pathway and how they could be targeted to treat insulin resistance.

Conflict of interest

The authors declare that they have no conflict of interest.

References

- Accili D, Arden KC (2004) FoxOs at the crossroads of cellular metabolism, differentiation, and transformation. *Cell* **117**: 421–426
- Brunet A, Bonni A, Zigmond MJ, Lin MZ, Juo P, Hu LS, Anderson MJ, Arden KC, Blenis J, Greenberg ME (1999) Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. *Cell* **96**: 857–868
- Cai D, Yuan M, Frantz DF, Melendez PA, Hansen L, Lee J, Shoelson SE (2005) Local and systemic insulin resistance resulting from hepatic activation of IKK-beta and NF-kappaB. *Nat Med* **11**: 183–190
- Dowell P, Otto TC, Adi S, Lane MD (2003) Convergence of peroxisome proliferator-activated receptor gamma and Foxo1 signaling pathways. *J Biol Chem* **278**: 45485–45491
- Fan W, Morinaga H, Kim JJ, Bae J, Spann NJ, Heinz S, Glass CK, Olefsky JM (2010) FoxO1 regulates Tlr4 inflammatory pathway signalling in macrophages. *EMBO J* **29**: 4223–4236
- Fessler MB, Rudel LL, Brown JM (2009) Toll-like receptor signaling links dietary fatty acids to the metabolic syndrome. *Curr Opin Lipidol* **20**: 379–385
- Hotamisligil GS, Shargill NS, Spiegelman BM (1993) Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science (New York, NY)* **259**: 87–91
- Puigserver P, Rhee J, Donovan J, Walkey CJ, Yoon JC, Oriente F, Kitamura Y, Altomonte J, Dong H, Accili D, Spiegelman BM (2003) Insulin-regulated hepatic gluconeogenesis through FOXO1-PGC-1alpha interaction. *Nature* **423**: 550–555
- Solinas G, Vilcu C, Neels JG, Bandyopadhyay GK, Luo JL, Naugler W, Grivnickov S, Wynshaw-Boris A, Scadeng M, Olefsky JM, Karin M (2007) JNK1 in hematopoietically derived cells contributes to diet-induced inflammation and insulin resistance without affecting obesity. *Cell Metab* **6**: 386–397
- Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante Jr AW (2003) Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* **112**: 1796–1808
- Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, Sole J, Nichols A, Ross JS, Tartaglia LA, Chen H (2003) Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J Clin Invest* **112**: 1821–1830