



PAPER

Insulin-regulated transcription factors: molecular link between insulin resistance and cardiovascular risk factors

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Patients with insulin resistance and/or type 2 diabetes have a 5-fold increase in cardiovascular mortality rate. Therefore, it is a current issue of discussion that arterial hypertension, lipid disorders as well as visceral obesity are coronary risk factors, which might belong to a syndrome that is caused by decreased insulin sensitivity. Concerning a possible molecular link between insulin resistance, atherosclerosis and obesity, we focus in our research on questions looking for a molecular link between lipid metabolism, insulin action, and obesity at a gene regulatory level.

Alterations in the structure, function and regulation of transcription factors appear to be such signalling steps which might play an essential role in the pathogenesis and therapy of cardiovascular risk factors associated with insulin resistance, eg the so called metabolic syndrome. Recent examples are members of the nuclear hormone receptor superfamily, eg peroxisome proliferator-activated receptor (PPAR) isoforms and sterol regulatory element-binding proteins (SREBPs). Beside their regulation by different metabolites, these transcription factors are also targets of hormones, like insulin and leptin, growth factors, and inflammatory signals. Therefore, they appear to be a point of signalling convergence at a gene regulatory level.

Major signalling pathways coupling receptors at the cell surface for hormones, growth factors as well as cytokines to gene regulatory events in the nucleus are the MAP-kinase cascades. We have recently defined different postreceptor defects in these pathways in patients with clinical phenotypes corresponding to congenital lipoatrophy. Therefore, these studies may identify novel pathways which play a role in the control of body weight, insulin sensitivity and cardiovascular risk.

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Introduction

Cardiovascular risk in patients with diabetes mellitus is increased 3- to 5-fold. Hafner *et al* have shown recently, that diabetic patients without previous myocardial infarction have a similar risk of myocardial infarction as non-diabetic patients with previous myocardial infarction.¹ This study compared the 7-y incidence of cardiovascular mortality among 1373 non-diabetic subjects with the incidence among 1950 diabetic subjects in Finland. Therefore, it is a current issue of discussion, that type 2 diabetes and coronary heart disease have some common predisposing environmental and genetic factors in their pathogenesis. Several recent studies indicate that arterial hypertension, lipid disorders as

well as visceral obesity are coronary risk factors which might belong to a syndrome that is caused by decreased insulin sensitivity with consequent hyperinsulinaemia called 'metabolic syndrome'. In the following we will give some insights into examples of transcription factors, controlled by MAP-kinase cascades which might play a role in the development of the metabolic syndrome, including fat distribution and control of body weight.

Congenital lipoatrophy

The genetic basis of insulin resistance, diabetes and coronary heart disease is very heterogeneous. One reason for this 'genetic nightmare' of the metabolic syndrome is that its main components, such as hypertension, dyslipidaemia, obesity, and diabetes are not defined or clinically classified by common essential pathogenic features, but rather

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arbitrarily by certain cut-off-points of physiological variables, ie levels of blood pressure, plasma levels of lipids or blood sugar and body weight. However, it is most likely that a diverse set of genes, rather than a single gene, determine whether the degree of decreased insulin sensitivity or action leads to clinically detectable blood sugar elevation and/or other features of the metabolic syndrome. Different genetic approaches have been undertaken to identify gene loci in multifactorial diseases, all of which might play a complementary role in the overall picture, eg linkage analysis, association studies, positional cloning, generation and investigation of genetically altered mice and characterization of patients with rare genetic syndromes. The value of the latter approach has been proven by the identification and characterization of the LDL-receptor pathway in patients with familial hypercholesterolaemia.² These studies using cultured fibroblasts of patients with homozygous and heterozygous familial hypercholesterolaemia have identified the LDL-receptor as a major candidate gene locus for the multifactorial disease process leading to coronary artery disease. In these patients several hundreds of different mutations in the LDL-receptor gene have now been identified.³ Each of these mutations is apparently very rare, but these studies have paved the way to the understanding that the LDL-receptor is also a major pathway for regulatory defects in patients with different forms of polygenic and dietary induced hypercholesterolaemia. In addition, this pathway has become the drug target of statins, which appear to be one of the most successful drug groups in modern cardiovascular medicine.

In analogy, to understand the molecular basis of body weight control, different genetic syndromes with severe obesity have been investigated and various mutations in a spectrum of candidate genes have been identified.⁴ We are investigating cultured fibroblasts of patients with the other extreme clinical phenotype, ie congenital lipoatrophy. There is recent evidence in some forms of congenital lipoatrophy that genetic defects affecting nuclear events might be involved.⁵ Furthermore, transgenic mice with mutations affecting the DNA binding of B-ZIP transcription factors of both the C/EBP and Jun families, which are also regulated by MAP-kinase cascades, exhibit a complete lack of white adipose tissue.⁶ This lipoatrophy is associated with insulin resistance and lipid disorders. These and other studies indicate that complex gene regulatory events controlled by transcription factors regulated by MAP-kinase cascades might be signalling pathways which are involved in the control of insulin sensitivity as well as body weight and may be altered in patients with lipoatrophy.

Postreceptor defects of the MAP-kinase cascades in patients with lipoatrophy

Protein phosphorylation at serine and treonine residues is a key regulatory mechanism controlling proteins regulating metabolism, growth, differentiation, apoptosis and gene expression of cells.⁷ A major class of serine/treonine kinase

mediating signal transduction of various extracellular stimuli, including insulin and growth factors, are mitogen-activated protein kinases (MAPK). Pathways involving MAPK consist of three kinases: MAP-kinase kinase kinase (MKKK); MAP-kinase kinase (MKK); and MAPK, which are sequentially activated.⁸ The activity of MAPK is stimulated by MKK-mediated dual phosphorylation (Thr-X-Tyr) in the activation loop. MKK is regulated by the serine/treonine kinase MKKK, which is linked by protein-protein interaction phosphorylation or subcellular relocation to extracellular stimuli at the cell surface, similar to the receptor-associated tyrosine kinases of insulin and growth factors. Different MAPK cascades have been identified, but the best characterized in mammalian cells is the extracellular signal-regulated kinase (Erk) pathway leading to the activation of the MAPK isoforms Erk-1 and Erk-2. We have focused to identify inherited postreceptor defects affecting this MAP-kinase cascade in cultured fibroblasts of patients with syndromes of insulin resistance and lipoatrophy.⁹⁻¹¹ We have found signalling defects in different patients at various levels of the Erk-MAPK cascade, which might be related to the degree of clinical insulin resistance in some of these individuals. Furthermore, these and other studies indicate that several defects may exist in a single patient at the postreceptor level, thereby affecting not only the action of insulin, but also of IGF-1 as well as other growth factors. This shows that defects in the MAP-kinase cascade can be associated with decreased cellular insulin sensitivity and probably complex gene regulatory events. The majority of identified MAPK substrates are transcription factors regulating the expression of many genes.¹² Transcription factors phosphorylated by activated Erk-1/2 are involved in hormone action, cell growth and differentiation, including PPAR γ ,^{13,14} and sterol regulatory element-binding proteins (SREBPs).¹⁵⁻¹⁷

SREBPs: potential gene regulatory targets for the metabolic syndrome

Sterol regulatory element-binding proteins (called SREBP-1a, SREBP-1c and SREBP-2) are transcription factors, that appear to transmit the signal of membrane-embedded cholesterol levels to the nucleus regulating the expression rate of multiple genes.^{18,19} Recently, we have provided evidence that SREBPs are not only involved in cholesterol-regulated events, but are also targets of intracellular signalling pathways, eg Erk-MAPK cascades.^{15-17,20} We have shown, that the effects of insulin and PDGF on LDL-receptor promoter activity are abolished by a MAP-kinase inhibitor and are mediated via the SREBP-binding cis-element *sre-1*. Overexpression of constitutively active upstream members of the Erk-MAPK cascade, like MEK-1/2, lead to a *sre-1* and SREBP-related stimulation of LDL-receptor promoter activity. Recently, we have shown, that SREBPs are direct substrates of Erk-1 and Erk-2, *in vitro*. Using protein chemistry methodology, like anion exchange chromatography, reverse-phase HPLC, and mass spectrometry as well as Edman degradation, we have

identified serine 117 as a major phosphorylation site of Erk-1/2 in SREBP-1a, *in vitro*. Mutation of serine 117 to alanine abolishes the SREBP-1a-mediated effect of insulin and PDGF on promoter activity. In accordance to that, we have identified multiple phosphorylation sites in SREBP-1c, which also appears to be involved in insulin action. SREBP-1c is the rat homologue to ADD (adipocyte determination- and differentiation-dependent factor)-1, which was cloned by the group of Spiegelman as an adipogenesis related transcription factor; its overexpression in fibroblasts promotes adipocyte differentiation and increased transcriptional activity of PPAR γ .²¹ Therefore, ADD1/SREBP-1c seems to be a link between cholesterol and lipid metabolism, adipogenesis, as well as insulin sensitivity. Accordingly, transgenic mice overexpressing ADD1/SREBP-1c in white adipose tissue exhibit cardinal clinical features of lipotrophic diabetes.^{22,23}

Perspective

Discovery and elucidation of complex gene regulatory networks will ultimately lead to the identification of master regulators like transcription factors, which will give new insights into the pathophysiology of complex clinical phenotypes like obesity, and provide medicine with new potential drug targets. Therefore, the relationship between structure and function of these transcription factors, their regulation, gene targets as well as the role in different cells or tissues have to be understood.

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