Editorial comment

Genetics and molecular biology: peroxisome proliferator-activated receptor γ still full of surprises

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Abbreviations

PPAR	peroxisome proliferator-activated receptor
RXR	retinoid X receptor

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About 10 years ago, a rodent nuclear receptor was identified on the basis of its ability to be activated by so-called peroxisome proliferators, a diverse group of hepatocarcinogens that includes various hypolipidemic drugs, plasticizers and herbicides. This ligand-activated transcription factor was therefore named the peroxisome proliferator-activated receptor (PPAR) [1]. Since then, the field of PPAR research has exploded, so that this name is no longer very helpful. Three main types of PPAR (α, γ and δ) have been described in humans with diverse and sometimes antagonistic roles in intermediate metabolism, in control of the inflammatory response and in cell differentiation.

For lipid specialists, much interest has focused on PPARγ. This receptor is expressed mainly in fatty tissue, but also in the gut, macrophages and, to a small extent, in muscle. It functions as a heterodimer with the retinoid X receptor (RXR) and has a basically anabolic function, stimulating lipid synthesis, fat storage and adipocyte differentiation. Indeed, Johan Auwerx has called PPARγ the 'ultimate thrifty gene'.

From a therapeutic perspective, PPARγ has been identified as the target for the thiazolidinediones, a class of antidiabetic drugs that improves insulin sensitivity, increasing peripheral glucose utilization and reducing endogenous glucose production. The molecular mechanism by which thiazolidinediones act on glucose metabolism, however, is not known. Fat is replete with PPARγ, but this tissue does not participate in glucose elimination to any significant extent, while muscle, the main site of glucose use, has little PPARγ, as already noted above. Several hypotheses have been advanced to explain this paradox, for example, that the low level of PPARγ expression in muscle might suffice to stimulate muscular glucose utilization, or that thiazolidinediones might induce PPARγ in muscle. In addition, thiazolidinediones have been shown to reduce the secretion by adipose tissue of tumor necrosis factor α and leptin, both factors that interfere with the insulin signalling cascade. Finally, it has been suggested that thiazolidinediones might selectively increase the uptake of fatty acids by adipose tissue, reducing their availability to muscle, thus leading via the Randle mechanism to an increase in muscular glucose utilization. In addition to the lack of clear information on the mechanism of action of thiazolidinediones on glucose metabolism, there is also no clear explanation as to why these drugs tend to cause weight gain, a side effect that partially cancels the benefit of tighter glucose control.

A recent paper by Yamauchi *et al.* [2] sheds new light on both of these PPAR puzzles. These researchers were intrigued by the surprising observation of themselves and other groups that, in mice and humans, genetic defects leading to a modest reduction in PPARγ activity protected against the insulin resistance and obesity induced by a high-fat diet. They therefore asked if pharmacological inhibition of PPARγ or RXR might also be of use in the treatment of obesity and type II diabetes mellitus. Yamauchi and co-workers used two mouse models of obesity-linked diabetes, KKAy mice [lethal *yellow* obese mice of the Japanese KK strain with a dominant allele at the *agouti* locus (A^y/+) leading to overexpression of the agouti peptide, which acts in the brain to increase feeding behaviour], and *db*/*db* mice, which bear a deletion in the long isoform of the leptin receptor and which are therefore unable to respond to the satiety signal leptin. Nonobese, nondiabetic C57 mice served as controls. These mice were treated with the synthetic RXR antagonist HX531 and with the PPARγ inhibitor bisphenol A diglycidyl ether. In a very dense study (the paper contained one table but no fewer than 50 graphs and figures), Yamauchi concluded that moderate downregulation of PPARγ has three main effects. First, molecules involved in triglyceride accumulation are reduced due to a fall in the expression of

PPARγ target genes such as fatty acid translocase. Second, levels of leptin are increased. This leads to a leptin-induced suppression of genes encoding proteins involved in lipogenesis such as sterol response element binding protein 1, stearoyl coenzyme A desaturase 1 and, again, fatty acid translocase. Third, downregulation of PPARγ appears to produce a secondary increase in PPARα activity, which increases the expression of proteins that produce energy dissipation such as uncoupling protein 2 and acyl coenzyme A oxidase.

Overall, Yamauchi's data indicate that the principal role of the PPARγ/RXR heterodimer might be the partitioning of fuel among tissues, and that the relationship between insulin resistance and PPARγ/RXR activity might follow a U-shaped curve. At 'normal' levels of PPARγ/RXR activity, a high-fat diet causes increased influx of fatty acids into white adipose tissue, muscle and liver, leading to increased storage of triglyceride in these tissues. In addition, this level of PPARγ/RXR activity constitutively suppresses leptin expression, which contributes to the development of obesity. Both of these effects lead to insulin resistance. Moderate reductions in PPARγ/RXR activity reduce the influx of fatty acids to white adipose tissue, liver and muscle, even in the presence of a high-fat diet. Also, expression of leptin is no longer suppressed, leading to decreased food intake and decreased expression of lipogenic proteins, as noted above. PPARα activity is also increased in liver, brown adipose tissue and muscle with increased energy dissipation. These three effects combine to limit development of obesity and insulin resistance. Marked reductions in PPARγ/RXR activity, however, have deleterious effects, channelling free fatty acids almost exclusively to muscle tissue and away from fat. This leads to a severe depletion of white adipose tissue, which then secretes little or no leptin. This in turn leads to increased expression of lipogenic proteins. In addition, the virtual absence of adipose tissue blunts the energy-dissipating effects of PPARα activation. The increased triglyceride storage in muscle and liver causes insulin resistance in the face of lipoatrophy. Yamauchi and colleagues go on to suggest that the main pharmacologically relevant effect of supra-physiological stimulation of PPARγ activity might be stimulation of adipocyte differentiation, leading to increased flux of fatty acids to fat and away from muscle and liver, improving insulin sensitivity at the cost of obesity.

Certainly, this is a report that will stimulate much discussion. While some methodological questions remain, for example concerning the specificity of the pharmacological mediators used, there is no doubt that this paper is an important contribution to our understanding of the complex web of gene expression regulating energy balance in humans.

The multi-faceted role of PPARγ in physiology is also highlighted by an interesting report from Tarrade *et al.* [3] on the effect of the PPARγ/RXRα heterodimer in controlling trophoblast invasion in humans. These workers found that in the human placenta during the first trimester, PPARγ and RXRα are highly expressed in the extravillous cytotrophoblasts. These are the cells at the tips of the placental villi that actively and rapidly invade the uterus and that are in direct contact with the maternal blood. Moreover, in a cell culture model, stimulation of this heterodimer by agonists for PPARγ and RXR inhibited cytotrophoblast invasion in a dose-dependent fashion. Antagonizing PPARγ using bisphenol A diglycidyl ether or inhibiting RXR using the RXR antagonist RO26-505 had the opposite effect. One implication of these findings is that the administration of PPARγ activators in early pregnancy might reduce trophoblast invasion and lead to complications such as pre-eclampsia, which has been linked to defective invasion of the spiral arteries of the uterus. Unfortunately, the authors of the study do not address this point.

Taken together, these two reports indicate that PPARγ is still full of surprises and that the pharmacological modification of its activity may produce unexpected effects – and side effects.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:
- • of special interest
- •• of outstanding interest

1 Green S. Peroxisome proliferators: a model for receptor mediated carcinogenesis. Cancer Surv 1992; 14:221–232.

2 Yamauchi T, Waki H, Kamon J, *et al.* Inhibition of RXR and PPARgamma ameliorates diet-induced obesity and type 2 diabetes. J Clin Invest 2001; 108:1001–1013.

3 Tarrade A, Schoonjans K, Pavan L, *et al.* PPARgamma/RXRalpha heterodimers control human trophoblast invasion. J Clin Endocrinol Metab 2001; 86:5017–5024.

Recommended reading

Fukumoto Y, Libby P, Rabkin E, *et al*. Statins alter smooth muscle cell
• accumulation and collagen content in established atheroma of watanabe heritable hyperlipidemic rabbits. Circulation 2001; 103:993–999.

It is thought that the collagen content of the atherosclerotic plaque affects its stability. In addition to their lipid-lowering capacity, inhibitors of the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A reductase (statins) are known to exhibit a variety of diverse, or 'pleiotropic', effects on atherosclerosis, one of which is alteration of the composition of the interstitial matrix of the lesion. In this report, the authors show that both pravastatin, a hydrophilic statin, and fluvastatin, a cell-permeable lipophilic statin, decreased the expression of matrix metalloproteinases 1, 3 and 9 by macrophages in the arterial intima. At the same time, the amount of interstitial collagen present in the plaque, which is presumably a marker of plaque stability, remained stable on pravastatin, but fell on fluvastatin. This tied in with the observation that fluvastatin reduced the number of human aortic smooth muscle cells, a major collagen source, in an in-vitro assay, although the mechanism of this effect was not resolved. The implications of these results for plaque rupture are unclear. Although the dose of fluvastatin used was above the therapeutic range in humans, these results signal potential danger with this agent. As the authors cautiously, if somewhat cryptically, note, these findings 'provide further important information for development of more effective therapies for atherosclerosis'.

Halene S, Kohn DB. Gene therapy using hematopoietic stem cells: Sisyphus
•• approaches the crest. Hum Gene Ther 2000; 11:1259–1267.

In animals, transplantation of genetically altered, allogenic hemopoietic stem cells has been successful in treating a range of inherited hemopoietic and lymphoid disorders. However, the task of gene transfer into human hemopoietic stem cells has proved to be far more difficult. Progress has been disappointingly slow and initial clinical trials failed to achieve significant gene marking. Various delivery systems have been used, including naked DNA, liposomes, adenoviral vectors, adeno-associated virus vectors, retroviruses, and HIV-1-based lentiviruses. Several transduction modalities and treatment of cells with cytokines and vector envelopes have also been tested, but to no avail. In addition, stable and specific expression of the gene of interest at the right level in the right tissue has not yet been achieved, and immunogenicity of the transferred gene product is still a problem. Clinical trials have shown little efficacy. Although there has been an occasional flicker of light on the horizon, it would appear that the dawn of gene therapy for hemopoietic therapy, like that of gene therapy for coronary restenosis (see below), is still far off. The authors chose a somewhat unfortunate title for this paper. Recall what happened when Sisyphus reached the crest of the hill: the boulder he had been pushing rolled back down to the bottom, so he had to start again from scratch. Let us hope that workers in the field of gene therapy will be spared that bleak fate.

Murphy EA, Waring AJ, Murphy JC, *et al*. Development of an effective gene
• delivery system: a study of complexes composed of a peptide-based amphiphilic DNA compaction agent and phospholipid. Nucl Acids Res 2001; 29:3694–3704.

This paper describes a system that transfected cell lines *in vitro* with an efficiency comparable to that of the commercial transfection agent Lipofectamine or polyplex polyethylenimine. As the authors themselves admit, however, this system is a long way off from becoming a tool for use *in vivo*.

O'Sullivan M, Bennett MR. Gene therapy for coronary restenosis: is the
•• enthusiasm justified? Heart 2001; 86:491–493.

Despite advances in techniques, restenosis of the coronary artery following percutaneous dilatation remains an important cause of morbidity. In the heady 1990s, gene therapy was a shining hope offering the promise of permanent antistenotic treatment. However, hard reality has now reasserted itself, as is clearly described in this excellent review. Two major questions must be answered before gene therapy of coronary restenosis becomes a reality: What genetic material should we deliver? and How can we deliver this material effectively and safely? With regard to the first question, most of the gene therapy studies that have been performed to date were done in animals. In animal models, gene therapy has been used to modulate the migration of arterial smooth muscle cells and cells of the adventitia, to manipulate smooth muscle cell proliferation and apoptosis, and to affect matrix deposition and medial and adventitial fibrosis. It has not been possible, however, to transfer these results to humans. In addition, neointimal response to injury varies between species. With regard to the question of delivery, many catheter systems have been developed, but most have proved to be inefficient. Moreover, needle catheters with facilitated intraluminal gene transfer may exacerbate vascular injury. For these reasons, coated stent technology appears to offer the better choice at the present time. Finally, a suitable gene transfer vector has yet to be found. Plasmid liposomes and retroviruses suffer from low gene transfer efficiency, transient transfection, and provocation of local inflammatory response. Safety concerns also exist, particularly with the use of retroviruses. All in all, the authors draw the gloomy conclusion that we may not expect gene therapy of coronary restenosis to be a viable option in the foreseeable future.