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PPARγ and the control of adipogenesis

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Summary — We recently cloned PPARγ as a factor that binds to an enhancer which has specificity for adipose cells. When expressed ectopically, PPARγ converts fibroblasts into *bona fide* preadipose cells. Upon application of activators or PPARγ ligands, these cells differentiate into fat cells. Most recently, we have been trying to understand the nature of natural ligands that activate PPARγ and the protein domains that control adipogenesis. With regards to ligands, we have shown that an unusual prostanoid, 15-deoxy$\Delta^{12,14}$PG J2, can bind to PPARγ and activate it. A second transcription factor that is induced early in differentiation, ADD1/SREBP1, appears to promote the formation of PPARγ ligands. Transfection of this molecule, a member of the bHLH family, causes the secretion of molecules that can serve as ligands for PPARγ. This ligand-like activity is specific for the γ isoform of PPAR. Current studies are attempting to identify these potentially novel ligands. With regard to structure-function of PPARγ, we first analyzed the adipogenic activity of the three isoforms of PPAR: α, γ and δ. Using appropriate activators of each it is clear that PPARγ has the most adipogenic action. PPARα can be adipogenic with high levels of the strongest activators and PPARδ does not stimulate fat cell differentiation. To identify the domain(s) of PPARγ responsible for differentiation, chimeras between PPARγ and PPARδ were created and transfected into fibroblasts. This has allowed the isolation of relatively small regions of this molecule that are responsible for differentiation.

PPAR / adipocytes / adipogenesis / preadipose cells

Introduction

A few years ago it was thought that adipocytes had solely a storage role in lipid metabolism. It now appears that they have a more dynamic role in integrating the status of energy balance. In the seventies, adipogenesis was mainly studied by endocrinologists. We are now working at the molecular level using the adipocyte protein aP2 as an adipocyte marker.

Two years ago we cloned PPARγ from a fat library and isolated a specific isoform called PPARγ2, which has a 30 amino acid extension, as compared to PPARγ1. PPARγ is mainly expressed in fat (50-fold higher than in other tissues): it seems to be adipose selective, while PPARα is mainly expressed in liver, kidney and heart and PPARδ in brain, heart and kidney [1].

Results and discussion

The first functional question we wanted to answer was: is PPARγ in presence of ETYA and RXR sufficient to activate fat selective enhancer for expression in other cells, such as fibroblasts for example? RXRα and PPARγ could activate fibroblasts: they are sufficient to activate a fat enhancer.

The next question is the following: can PPARγ play a role in adipose differentiation? Using a retrovirus system, Tontonoz *et al* tried to see if one could get adipogenesis in cells that usually did not give any. With RXRα and PPARγ as vectors, ETYA give an abundant differentiation, though there is no overexpression protein (only 20–30% expression seen in normal adipocyte cells). It is not the only transcription factor involved in adipose differentiation though [2]. With C/EBPα and PPARγ together, without using ligand or activator, one gets a very reasonable differentiation. There seems to be a synergistic activation between C/EBPα and PPARγ.

PPARγ having a very high homology with other PPARs, it seemed interesting to see if other PPARs were adipogenic. With γ expression, almost any compound that has an activator activity towards γ can induce adipogenesis.

PPARα can get a *bona fide* adipose differentiation, but only with strong activators.

PPARδ, in our hands, never gave adipose differentiation [3]. PPARγ plays an important role in adipogenesis, but it is important to understand the balance between cell growth and cell differentiation: too much proliferation with poor differentiation leads to a poorly organized embryo or perhaps tumorgenesis.

Many hormones and growth factors have an impact on adipogenesis. In standard preadipocyte cell lines, insulin has a positive role on adipogenesis, in contrast to the usual role of growth factor which are good suppressers of adipogenesis like PDGF, EGF, FGF, TGFβ or the tumor promotor TPA. However, in fibroblasts overexpressing insulin receptors, insulin acts as growth factor and does not cause differentiation.

In cells transfected with PPARγ, treated 30 min with growth factors, and after doing a Western blot with anti-

PPARγ, one can observe a mobility shift from a lower to an upper form, except with TNFα. This mobility shift suggests a phosphorylation, reversible by phosphatase, as confirmed by our experiments. A treatment by phosphatase shifts down to the lower γ form [4].

By ^{32}P labeling, most of the ^{32}P radioactivity is associated with the higher form of PPARγ in insulin-treated cells. It appears that there is an insulin-associated increase of phosphorylation. This effect is abolished by treatment with phosphatase.

Using this mobility shift and deletion, we tried to map the site responsible for this mobility shift. It appears the serine at 112 site is a consensus recognition site for serine/threonine kinase.

We could reproduce PPARγ phosphorylation with an *in vitro* assay using MAP kinase and we created a mutant PPARγ with an ala site which blocks the mobility shift and gave confirmation that this site, as well as MAP kinase or members of its family, were involved in the phosphorylation process in cells [4].

The next question was: what is the effect of phosphorylation on transcriptional activity and behavior of cells in differentiation? At first, there was no change in the location of PPARγ by phosphorylation and RXR with mutant transactivation assay. In bioassay, activation of MEK (which is a kinase upstream of the MAP kinase) allows to suppress the PPARγ2-ligand transcriptional activity of the wild type in a much greater extent than the mutant. Phosphorylation of PPARγ can be also inhibited by MAP kinase inhibitors like forskolin, 8-β-CAMP but also by specific inhibitors of MEK kinase which is upstream of MAP kinase [4].

In cells treated with TPA and dexamethasone, the transactivation suppression of wild type was of about 70%, with marginal effects on mutant. It appears that the site is involved in the suppression of transcriptional activity using a DR1 reporter.

With differentiation assays, it appeared that the mutant dramatically sensitizes the process of differentiation. By knocking out the kinase input in the 112 site, there is a 1 or 2 orders of magnitude shift in the dose-response in the ability of PPARγ ligands (pioglitazone) to induce adipogenesis. Other assays (stained dishes) also revealed that the triglyceride accumulation in pioglitazone saturating conditions took off earlier in mutant cells [4].

These results indicate that the PPARγ2 ala mutant increases both the rate and the extent of differentiation and reveal that PPARγ2 and MAP kinase are the real components of the process.

Also, though many growth factors are suppressors of adipogenesis, with the mutant, the cells are resistant to the suppression of differentiation by TPA or FGF as shown by mRNA levels of AP2 and adipsin.

Conclusion

It seems as if the MAP kinase enzymes strike the PPARγ in one site and turns off differentiation. An increase in MAP kinase activity reduces PPARγ activity, thus differentiation. Differentiation in mutant cells becomes almost spontaneous. There is a balance between growth and differentiation where PPARγ is an important transcription factor. A low activity of PPARγ gives a low level of differentiation. Growth factors modify signaling of PPARγ of the adipose cell differentiation process to suppress the ability of PPAR to differentiate the cells, where MAP kinase is an extremely important player. Adipose differentiation appears to be more ready to occur than was thought of, and its is essentially the role of the growth factors to keep the whole system quiet.

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