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The role of PPAR gamma in the immune system with a systems biology approach: A most read overview of transcription regulation in immune cells

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Abstract

Systems biology approaches have become indispensable tools in biomedical and basic research. These data integrating, bioinformatics methods gained prominence after high-throughput technologies became available to investigate transcription regulation, protein-protein interactions, gene silencing among other biological processes. Immunology is one of the medical fields that systems biology impacted profoundly, due to the plasticity and the accessibility of experimental models. In this review we will summarize the available information on Peroxisome proliferator-activated receptor gamma (PPAR γ) genome wide studies, and other high-throughput methods in a group of innate immune cells: macrophages and dendritic cells. We will contrast the knowledge obtained with traditional methods and state-of-the-art techniques in these immune cells regarding the function of PPAR γ .

Introduction

Our body is protected against intruders by complex mechanisms. Multiple layers of defense exist, which are part of the highly ordered, hierarchical and integrated immune system. Cell types involved in the immune response include among others various types of T-cells, B-cells, Natural Killer (NK) cells, dendritic cells and macrophages. Decades of research have led to the identification of several key molecular components that define the functions and downstream effects of these immune cells, however our understanding is still far from complete.

Importantly, traditional research strategies lacking high-throughput technologies ignored the complexity of biological systems, screening gene functions on individual targets rather than focusing on biomolecular networks. Introduction of novel systems biology approaches opened the way to investigate the consequences of manipulation of a gene on the entire molecular network and has revolutionized the way we think about biological systems and their complexity.

In this review we will summarize the recently available PPAR γ -related “omics” data in the context of different immune cell types to demonstrate how one single protein can play such a diverse role in multiple biological processes. Moreover, we will demonstrate that with the re-analysis of existing genome-wide studies one can get

further insights into the biological role of a factor and identify novel cellular signaling pathways.

Systems biology approaches in immunology research.

Systems biology is the study of a complex entity using a holistic approach; which enables the prediction of the system behavior through the complex relationships among its components [1]. Such prediction of a system's behavior can and should be later validated experimentally. It is clear that a biological signal is not generated by a particular molecule alone, but instead by the complex interaction of several factors [2]. A main driving force behind holistic approaches has been the development of high-throughput methods, such as microarray, RNA-seq, proteomics, ChIP-seq, RNAi screens and 3D imaging. These techniques are now indispensable in biological and medical research. Analysis of data generated through these methods requires a multidisciplinary research team, which includes experts from medicine to mathematics [3].

In complex systems such as immunology networks, the use of a mathematical models and bioinformatics approaches holds a great potential in the identification of novel therapeutic targets for treating immunological diseases [4-6]. This approach also suites immunology studies in a sense that the cells can be isolated at various stages and the description of these stages will have a meaningful biological response [3].

Gardy et al., 2009 describe the system biology workflows available for immunology research, which includes the analysis of the transcriptome, the gene expression networks, proteomics, miRNAs, genetic polymorphisms and analyze the data in a network context [1]. Visualization of the high-throughput output information is also crucial for the understanding and analysis of the data. Advances in this segment can be already seen in the literature [7]. Published data can be re-analyzed in a variety of software; gene lists together with network generation can be obtained for further experimental research [8-10].

This approach in immunology can be used for clinical relevant outcomes such as identifying drugs that could treat immunological related diseases, but are used to treat other pathologies (drug repurposing). The data obtained from large datasets can be utilized to generate networks that associate drugs to genes and diseases [11]. PPAR γ

agonists have been inferred to have a therapeutic effect on colorectal cancer by systems biology and this effect has been proved in vivo experiments [12].

Genome-wide studies of transcription factor binding sites have been performed in immune cells specifically macrophages; these approaches pinpoint to the fact that lineage-determining transcription factors take part in defining the transcriptomic response upon stimulation [13].

PPAR gamma

PPAR γ is a ligand-activated transcription factor that directly binds to specific DNA sequences and can respond to various ligands [14, 15]. Many putative endogenous PPAR γ ligands have been identified, most of them derived from unsaturated fatty acids HETEs and HODEs, such as 9 and 13-HODE [16, 17]. PPAR γ appears to be a sensor of a group of oxidized lipids, too. Selective synthetic ligands of PPAR γ , called thiazolidinediones are also available, some of them being used in the treatment of diabetes [18].

PPAR γ is present mainly in adipose tissue and kidney and is essential in adipogenesis [19-21]. However, PPAR γ expression is not limited to metabolic tissues, it is also expressed in various cells of the immune system such as macrophages, dendritic cells, eosinophils, T cells and B cells [22-25].

PPAR γ forms a permissive heterodimer with RXR, another member of the nuclear receptor superfamily. The PPAR γ /RXR heterodimer can recognize specific sequences (PPAR response element (PPRE)) in the DNA, and can initiate transcription of a group of target genes.

Despite the large amount of data available regarding the role of PPAR γ in various model systems, it is safe to say that novel “omics” methodologies allow us a more systematic characterization and detailed investigation of PPAR γ function in different cell types. Transcriptomic data are already available and helped us to identify PPAR γ -regulated target genes in different immune cell types. Cistromic data are also available for PPAR γ and RXR and suggest a striking cell type-dependent binding pattern of PPAR γ /RXR heterodimer [26] (Figure 1). In the following sections we attempt to systematically summarize our recent knowledge of PPAR γ -mediated

macrophage and dendritic cell functions, including those novel insights that we could get with the application of “omics” technologies.

PPAR γ in Innate immune system

Innate immunity is the first line of defense in multicellular organisms [27]. The mononuclear phagocyte system represents a population of bone marrow-derived myeloid cells that can be found circulating in the blood as monocytes and populate tissues as macrophages in a resting state and during inflammation process. The importance of the Innate Immune system not only involves the genetic disorders, which affect the immune cells and therefore the immune system functions are impaired. In other conditions these extremely regulated cells are affected leading to co-morbidities [28].

The Macrophage and dendritic cells are principal players in the innate response against nocive signals [29]; they both come from a common committed progenitor cell in the bone marrow [30].

Macrophages

Macrophages participate in pathogen clearance and in the inflammatory response. The macrophage population is highly heterogeneous and is classified in subpopulations regarding their anatomical localization. PPAR γ has well documented accessory roles in the differentiation of the monocytes to macrophages in both mouse and human monocyte differentiation [31, 32].

PPAR γ in macrophages is involved in lipid metabolism but also in regulation of immune functions. As mentioned above, PPARs bind various fatty acids and fatty acids by-product, regulating aspects of lipid metabolism, differentiation and cellular growth by transcription regulation of several downstream genes [33]. PPAR γ natural and synthetic ligands inhibit the expression of inflammatory molecules [34-38]. Furthermore PPAR γ agonists inhibit the transcription of monocyte chemoattractant protein-1 (MCP-1) and its receptor CC chemokine receptor 2 (CCR2) in macrophages affecting the recruitment of macrophages to inflammatory sites [39-42]. Macrophages after the exposure of certain cytokines can be driven to classical or alternative activation.

Classical macrophage activation (resulting in so-called M1 macrophages) is driven by certain primary stimuli, such as INF gamma and as a second signal bacterial LPS or cytokine tumor necrosis factor- α , leading to an inflammatory phenotype secreting TNF α , IL-12, IL-1, IL-6 and NO [43, 44]; as result, M1 macrophages not only have phenotypic changes but also now they are capable of fighting bacterial infection [45]. On the other hand, PPAR γ has been implicated in alternative activation of macrophages mediated by IL4, leading to an anti-inflammatory phenotype (M2 macrophages). M2 macrophages have decreased levels of inflammatory cytokines and produce large amount of anti-inflammatory molecules including IL-10 and TGF- β [46]. In addition, IL-4 induces the expression of 15-lipoxygenase, autotaxin and monoamine oxidase and these enzymes produce ligands for PPAR γ [47, 48].

The proposed molecular mechanism, by which PPAR γ can represses pro-inflammatory cytokines, is called transrepression. Co-repressor complexes Ncor/HDAC3/TBL are bound to the promoter of inflammatory genes (i.e. iNOS); this association is disrupted when stimulation of LPS is performed. In contrast, in macrophages treated with PPAR γ agonists the repressor complexes remain in association with the promoter even after further treatment with LPS. This model includes the ligand-dependent SUMOylation of PPAR γ preventing the ubiquitination/proteasome-mediated degradation of the co-repressor complexes [49]. Nevertheless, this model is poorly understood and does not explain how PPAR γ can exert two different actions in the same cell type; in other words, how PPAR γ induce the dissociation of the co-repressor complexes from the targeted gene promoter in order to lead to gene expression and in the other hand, bind to the gene promoters that are required for transrepression [50].

Szanto et al., 2010 with a systematic genome wide transcriptome approach in human and mouse macrophages demonstrated that pro-inflammatory molecules inhibit meanwhile the treatment with IL-4 increases the expression of PPAR γ and its downstream target genes. It was also shown that the alternative macrophage activation could be achieved in the absence of PPAR γ using global gene expression analysis in PPAR γ deficient mice. Furthermore, the gene expression of alternative activation markers such as Ym1, Arg1, or Fizz1 was not affected upon rosiglitazone treatment. In addition, the authors could show in a genome wide analysis that IL-4 dependent PPAR γ induction is through facilitation by STAT6 [51].

Following this approach Scotton et al., 2005, Syed et al., 2007 and Czimmerer et al., 2012, distinguished IL-4 specific target genes in human macrophages with the aim to identify alternative macrophage activation markers. Applying microarray technology: CD1b/c/e, TLR1, and C-type lectin superfamily member 6, IL-1RI, IL-1RII, IL-1Ra, CCL17, CCL22, CCL23, CCL26, WNT5A, CD180, SLA, MS4A4A among others could be identified as novel markers. In silico analysis of the macrophage transcriptome during IL-4 treatment showed that IL-4 regulated genes were exhibited in immunological process; surprisingly, also were represented in cellular metabolism, cell proliferation and apoptosis. Transcription factor function results in the prediction of pathway specific transcription factors such as nuclear factor (erythroidderived 2)-like 2 as well as PPAR γ among others [48, 52, 53]

Martinez et al., 2013 provided a transcriptome and proteome analysis of human and mouse M2 macrophages. The comparison of resting and alternative activated macrophages in both species highlighted the transcripts and proteins expressed in resting macrophages and M2 macrophages, showed distinct cell type specific molecular signatures in each case. Nevertheless, 87 genes were found to be conserved and 50% of these genes were novel findings, including proteins involved in redox balance: glutathione S-transferase 1 (GSTO1) and glutathione peroxidase (GPX1). The cell type specific molecular signatures are visualized in different networks. Analysis of the functional and canonical pathways associated with them to functions comprising lipid storage, metabolism of xenobiotics, chemotaxis, and immune recognition. Importantly, transglutaminase 2 (Tgm2) was identified as a highly conserved M2 marker [54].

Genome-wide analysis of regulators regions occupied by PPAR γ in murine macrophages and the human macrophage like cell line THP-1 [55] revealed that human and murine PPAR γ binding sites are only 5% shared between the two species but PPAR γ downstream genes are conserved in 30% through evolution. As part of this analysis 4302 PPAR γ /RXR binding sites were found in THP-1 cells within this enriched sequences up to 60% were co-occupied with the well-established myeloid lineage specific transcription factor PU.1 [56], similar to the murine macrophage data. This led to the proposal of a model where PU.1 assist PPAR γ functional binding in macrophages throughout mammalian evolution.

Tissue specific macrophages have been shown to play an important role in disease; PPAR γ deficient mouse model has an impaired microbiota killing in colonic mucosa [57]. In silico analysis predicted macrophages as a therapeutic target in inflammatory bowel disease. Furthermore, PPAR γ deletion in these macrophages exaggerated clinical and pathological outcomes [58]. Global gene expression analysis of PPAR γ deficient colonic macrophages showed altered expression of 124 genes to control littermates. An IPA analysis of these genes showed two main pathways compromised: 1) carbohydrate metabolism and 2) immunological disease [59]. Previously by Mohapatra et al., 2010 down-regulation of lysosomal pathway genes were found in inflammatory bowel disease macrophages murine models. It appears that not only immunological processes are involved in macrophage PPAR γ signaling but also carbohydrate and lipid metabolic pathways, large-scale data analysis allow us to find other networks affected by PPAR γ □□□□□□□□□□.

A large-scale interconnection analysis has been performed in human and murine lupus nephritis renal biopsies. Berthier et al., 2012 used an algorithm called TALE (a Tool for Approximate Subgraph Matching of Large Queries Efficiently) in order to identify related networks affected in both species. Surprisingly in both species macrophage/DC related genes were affected. With this approach the authors could identify an enhancement of PPAR γ -dependent regulation in glomerular macrophages [60].

As stated above PPAR γ is involved in macrophage lipid metabolism [61]; through the PPAR γ response element in the promoter of the scavenger receptor CD36, this gene responds to PPAR γ activation [62, 63]. This receptor uptakes the oxidized low-density lipoprotein (LDL) in a cell type and context dependent manner [64]. In a murine model of mild and severe hypercholesterolemia the absence of PPAR γ exacerbated atherosclerosis; the protective mechanism of the receptor involved monocyte recruitment and efficient cholesterol efflux [65]. Also, inflammatory reactions in numerous cell types including endothelial cells can be inhibited using PPAR γ agonists [66]. These observations make PPAR γ an interesting target for prevention and treatment of atherosclerosis.

The early stages of atherosclerosis initiate oxidative modifications in the components of LDL complex, which drives the formation of fatty streaks in the endothelium of large arteries; as well as continuous macrophage accumulation with oxidized LDL

uptake that leads to the formation of atherosclerotic lesions [67]. It has been shown that non-thiazolidinedione PPAR γ agonists do not have anti-inflammatory effects [68]. Together with the results obtained by Chawla et al., 2001 where PPAR γ deficient macrophages responded to thiazolidinediones similarly than wild type macrophages inhibiting INF γ and IL-6 secretion suggesting that these drugs act as a PPAR γ independent manner. However, PPAR γ agonist inhibits the macrophage-foam cells formation in peritoneal cavity *in vivo* [69].

Atherosclerosis is reduced in LDL deficient murine models together with the decreased expression of inflammatory markers after the treatment with thiazolidinediones. Furthermore, atherosclerotic lesions are increased in PPAR γ null bone marrow transplanted LDL deficient mice [64, 70]. In the same *in vivo* model it has been observed that Rosiglitazone treatment reduced cholesterol accumulation in part independently from LXR [71].

Genome-wide study in human stable and rupture atherosclerosis plaques showed in a transcriptome level the PPAR/Adipocytokine signaling pathway mostly up-regulated in the ruptured plaques, among the genes up-regulated were FABP4 and Leptin [72]. These results show the importance of PPAR γ in the treatment and prevention of atherosclerosis, making it more important to continue the study of this receptor at a genome wide level during the formation of the lesions.

The presence of macrophages in adipose tissue confers an anti-inflammatory equilibrium during physiological conditions. This equilibrium can easily be disturbed by the development of pathological conditions such as obesity. This way, macrophages could be involved in metabolic processes at a systemic level in obesity.

An increase number of macrophages in the adipose tissue in obesity correlates with the body mass. These macrophages contribute in the adipose tissue expression of TNF α , IL-6 and iNOS in an extended measure. This conclusion was reached after the analysis of microarray experiments in obesity murine models [73]. Moreover these macrophages participate actively in the chronic inflammation in obese individuals, which finally will lead to insulin resistance and Diabetes Mellitus type 2 [74].

The function of PPAR γ in macrophages and adipocytes has similar pathways including lipid metabolism regulating genes including lipoprotein lipase, acetyl coenzyme A acetyltransferase and phospholipase A [75]. At the same time this receptor exhibits very different functions, which are cell type specific and involve

different signaling pathways in each cell type (Figure 1). In order to elucidate the reasons for this difference, a genome wide study through ChIP-seq technologies has been performed; Lefterova et al., 2010 investigated the differences between the binding sites in macrophages and adipocytes. The PPAR γ cistrome is cell type specific according to this study 30% of the regions overlap in the two cell types [26]. These data also support that tissue specific PPAR γ function is modulated by accessibility to the binding site by chromatin conformation, and tissue dependent transcription factors (e.g. PU-1 in macrophages, and C-EBP in adipocytes).

The gene networks involving PPAR γ differs between immune cell types as well. Depicting a very well orchestrate and highly specific gene regulation (Figure 2).

The need for genome-wide studies addressing this issue in macrophages and other cell types expressing PPAR γ are urgent.

Schneider et al., 2014 in a recent study found that PPAR γ is necessary for the differentiation from fetal monocytes into alveolar macrophages but not for other tissue-resident macrophages. The authors used microarray data to identify the transcription signature that PPAR γ grant to alveolar macrophages [31]. Lavin et al., 2014 using histone modifications ChIP-seq data in different population of macrophages identify more than 10,000 macrophage-specific enhancers. Furthermore, the enhancer map across the different macrophages is well marked and can be reprogrammed when precursors are transplanted in a different environment. They also found PPAR γ as a distinct signature for macrophages in the lung [76].

To decipher cell type specific functions a genome wide transcriptome and epigenome analysis in mouse knock out models and in human diseases is needed.

Dendritic cells

Dendritic cells (DCs) constitute a system of cells bridging innate and adaptive immune responses through their ability to orchestrate a wide range of lymphocyte responses according to signals perceived in the periphery. In most tissues DCs are present in an immature state, unable to stimulate T cells, but specialized to capture and process antigens. Various stimuli (e.g. microbial products, lymphocyte-derived signals, endogenous mediators) are capable of inducing a maturation process characterized by a switch from antigen-sampling to antigen-presenting and lymphocyte stimulatory activity, which requires enhanced surface expression of MHC

and costimulatory surface molecules (CD40, CD80, CD86). After migration to secondary lymphoid organs, activated DCs induce antigen- and tissue-specific adaptive responses. DCs also control the nature of T cell response by driving the differentiation of naïve T cells into different effector T cell subsets through exhibiting distinct secreted cytokine profiles. In addition to their role in adaptive immunity, DCs also elicit innate immune responses by producing cytokines involved in host defense, like IL-12 and interferons, and by activating NK and NKT cells [77]. In the course of their lifecycle, DCs encounter continuously changing microenvironments containing a diverse range of lipids. The lipid microenvironment can influence DC phenotype and therefore DC-induced immune responses via G-protein coupled receptors and nuclear hormone receptors. Among the latter, PPAR γ is increasingly appreciated as a sensor capable of translating lipid signals into gene expression changes, thereby shaping DC function.

Microarray analyses revealed that during *ex vivo* DC differentiation from human monocytes a group of genes involved in lipid metabolism is up-regulated. These genes encode proteins implicated in the production, uptake, transport and solubilization of cholesterol and fatty acids [78]. Several of them produce lipid metabolites capable of activating PPAR γ , such as monoamine oxidase A-produced serotonin metabolites or 15-lipoxygenase-produced eicosanoids [79, 80]. PPAR γ shows a marked and rapid up-regulation during this differentiation process, as well. Thus it seems that DC differentiation induces a switch of PPAR γ signaling pathway to a state when the receptor is, at least, ready to be activated by its intracellular ligands or lipids of extracellular origin, such as 15d-PGJ₂, LPA, or oxLDL [81] [82] [83]. Research from the last decade has shown that activation of this signaling pathway affects both the metabolism and immune phenotype of DCs [84]

The complexity of the downstream effects of PPAR γ activation has been revealed in its entirety by the global gene expression analysis of human monocyte-derived DCs differentiated in the presence of rosiglitazone for different time spans [85]. The results derived from this analysis indicated that PPAR γ does not act as a general inhibitor of DC development since the vast majority of genes regulated upon *ex vivo* DC differentiation showed unaltered expression in the presence of rosiglitazone. Rather, PPAR γ activation appears to skew DC differentiation creating a unique DC subset in terms of its transcriptional profile and consequential functional properties. Human serum enhances PPAR γ activity in *ex vivo* differentiated DCs, suggesting that

continuous low-level activation of PPAR γ signaling might be an integral part of the differentiation process *in vivo*. The dynamic temporal changes in PPAR γ -regulated genes reflect the two-faced role of the receptor in immune regulation, connecting metabolic and immune functions (Figure 3). After 6 hours, the category of lipid and fatty acid metabolism was markedly overrepresented among the genes up-regulated by rosiglitazone. These include genes involved in fatty acid oxidation (e.g. ACOX1, HADHSC), phospholipid and triacylglycerol synthesis (e.g. DGAT2, PPAP2B) and lipid uptake/transport (e.g. CD36, FABP4), showing that the receptor simultaneously regulates antagonistic metabolic processes. The net effect of these transcriptional changes seems to be the enhanced capacity of DCs to metabolize and redistribute lipids, as rosiglitazone-treated DCs accumulated fewer lipid droplets.

After 24 hours, the proportion of genes related to immune response showed a marked increase in the rosiglitazone-regulated genes. Among these, the CD1 group of surface proteins showed coordinate regulation by PPAR γ characterized by decreased expression of CD1a, CD1b, CD1c together with CD1e and elevated expression of CD1d molecules [83]. These MHC class I-like molecules are implicated in lipid antigen presentation to T cells. Group I CD1 molecules (CD1a, CD1b, CD1c) present self and microbial lipid antigens, whereas Group II (CD1d) molecules primarily bind self-lipid antigens [86]. In contrast to Group I CD1 molecules, which are recognized by TCRs showing diverse rearrangements, CD1d proteins are able to interact with T cells carrying invariantly rearranged TCRs, as well. These CD1d-restricted T cells are referred to as invariant natural killer T (iNKT) cells.[87]. Enhanced expression of CD1d in PPAR γ -instructed DCs endows them with the ability to selectively induce V α 24⁺V β 11⁺ iNKT cell expansion in the presence of certain lipid antigens [83]. iNKT cells have cytolytic activity and rapidly induce the expression of IL-4, IL-13 and IFN γ after TCR stimulation, which, in turn, can activate NK cells, T cells, B cells, macrophages, and recruit DCs. The rapid activation of iNKT cells potentially renders them powerful effectors of the early phases of immune response.

The regulation of CD1d expression and subsequent iNKT cell expansion by PPAR γ takes at least 24 hours to develop that suggested an indirect mechanism instead of direct transcriptional activity of the receptor. By studying the global gene expression profile of PPAR γ -activated human DCs Szatmari et al. found that activation of PPAR γ induces the retinoid pathway responsible for controlling CD1d expression. Specifically, activated PPAR γ induces the expression of retinal- and retinol-

metabolizing enzymes leading to increased intracellular generation of all-trans retinoic acid (ATRA) from retinol. ATRA activates RAR α receptor, which, in turn, induces CD1d transcription. ATRA-induced CD1d expression confers the ability to selectively activate iNKT cells [88].

Microarray analyses of DCs treated with the combination of rosiglitazone and an RAR α antagonist made it possible to determine, what portion of PPAR γ -regulated genes are controlled indirectly via induced retinoid synthesis and subsequent RAR activation. Unexpectedly, this analysis revealed that approximately 30% of PPAR γ ligand-responsive genes in DCs are regulated through the induction of the retinoid pathway [88].

Activation of PPAR γ in human monocyte-derived DCs also up-regulated several genes that are normally down-regulated during DC differentiation (e.g. PDK4, CD1d, TLR4, CD36, leukotriene B₄ receptor, transcription factor ATF3), possibly contributing to the development of a distinct PPAR γ -instructed DC phenotype. At the same time, 66.7% of genes, which were negatively regulated by rosiglitazone treatment were induced during DC differentiation (group I CD1s, IL1R1, IL1R2, IRF4, CD80, DCNP1), showing that the receptor inhibits certain aspects of the differentiation process [85].

PPAR γ forms a permissive heterodimer with RXR that can be potentially activated both by PPAR γ and RXR ligands. By comparing the transcriptome of human monocyte-derived DCs after specific PPAR γ and RXR activation, Szeles et al. found that only a subset of PPAR γ target genes possesses dual ligand regulation. The majority of these genes were regulated to a lesser extent by RXR activation, thus the permissiveness of PPAR γ /RXR heterodimer seems to be only partial in DCs [89].

Global gene expression profiling made it possible to decipher PPAR γ -regulated transcriptional changes throughout the genome and identify new lipid-responsive pathways that shape the functional properties of DCs. It is important to note though that studies on PPAR γ -regulated transcriptome were all carried out using *ex vivo* differentiated DCs. Thus, it would be crucial to extend studies on the cistrome and transcriptome of the receptor to DCs of *in vivo* origin in the steady-state of the immune system and also in the context of different disease challenges.

Future challenges of systems biology and perspectives

In the last four decades scientists identified many molecular components of a cell in the hope that it helps to understand how our body and various cells are working. Especially in the recent years, innovations, particularly in microarray and next generation sequencing technologies, and more recently mass spectrometry have made possible the generation of comprehensive dataset from a variety of biological systems. However, it has been realized in the last decade that listing the genes, looking for protein interactions and studying what tissues express which genes is not the whole story.

In this review we have attempted to present the emerging importance of systems biology in immunology in the context of PPAR γ . We have emphasized the novel findings achieved by integrating high- throughput data collection methods, such as microarray, RNA-seq, ChIP-seq to develop a deeper understanding of how the immune system operates at various scales and what is the importance of PPAR γ in this very complex process. Our summary of multiple function of PPAR γ in various cell types clearly demonstrate the existence of cross talk across multiple pathways, feed-forward and feed-back loops present in the PPAR γ network. Thus, representation of PPAR signaling pathway as linear cascades funneling signals from cell membrane to the nucleus is oversimplification of reality.

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Figure legends

Figure 1. Cell type specific of PPAR γ potential gene regulators. Reductionism point of view of PPAR γ molecular genetics in macrophages and adipocytes on gene Atp6v0d2 and Adipoq. B) IGV Browser snapshot showing genes Atp6v0d2 and Adipoq with PPAR γ , RXR ChIP-seq and RNA-seq data in macrophages and adipocytes (data obtained from Lefterova et al., 2010 Daniel et al., 2014, Nielsen et al., 2008 and Cuaranta-Monroy unpublished data)[26, 90, 91].

Figure 2. PPAR γ signaling networks in macrophages and dendritic cells. List of genes affected by PPAR γ published in The National Center for Biotechnology Information in macrophages (A) and dendritic cells (B) were generated by the on-line access software Genes2FANs. Next, using the same software networks for PPAR γ signaling were obtained in these two different cell types [10]. Nodes which were obtained from the seed list are colored in blue, the intermediated nodes in orange have a significant z-score and the gray nodes have a not significant z-score (significance cutoff = 2.5). Already published data can be used to generate gene lists and networks for identifying pathways in different cell types.

Figure 3. Proportional Venn diagram of rosiglitazone (RSG)-induced differences between control and RSG-treated samples showing the numbers of RSG-regulated genes at different time points ($p \leq 0.05$, fold change ≥ 2.0) (A). Top 5 Gene Ontology (GO) categories by p-value which were overrepresented among the genes regulated by RSG at different time points ($p \leq 0.05$, fold change ≥ 2.0) (B). Analysis is based on the microarray experiment of Szatmari et al. (GEO ID: GSE8658)[92].

BOX 1. Glossary of large-scale data acquisition technologies***Microarray***

Based on hybridization of complementary nucleic acid strands and one of the most widely used technology to identify dynamic transcriptomes in normal and disease states

gives poor performance in quantification of less abundant transcripts

Next generation sequencing (NGS)

Use massively parallel analysis of individually amplified DNA fragments and produces short (35-400bp) but tremendous amount (up to 10^7) of sequence reads

Target of the NGS analysis can be not only the genome, but methylated DNA, expressed mRNA (RNA-seq), DNase I hypersensitive DNA regions, or regions bound by a specific transcription factor (ChIP-seq)

ChIP-seq

Based on chromatin immunoprecipitation, where a protein specific antibody is used to purify DNA fragments that are associated with the protein of interest, for instance PPAR γ or its heterodimeric partner RXR. ChIP-seq is already revolutionized our understanding of how transcription factor binding and how epigenetic modifications control gene expression on a system level.

RNA-seq.

Through deep-sequencing of cDNA, this approach allow us to determine the expression levels of the whole transcripts and the isoforms at a given physiological or developmental stage. These transcripts include: non-coding RNAs, small RNAs and mRNA.

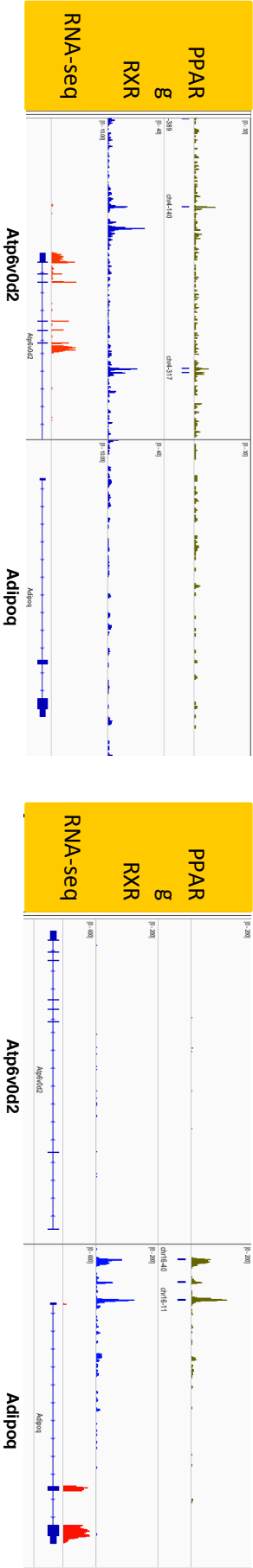
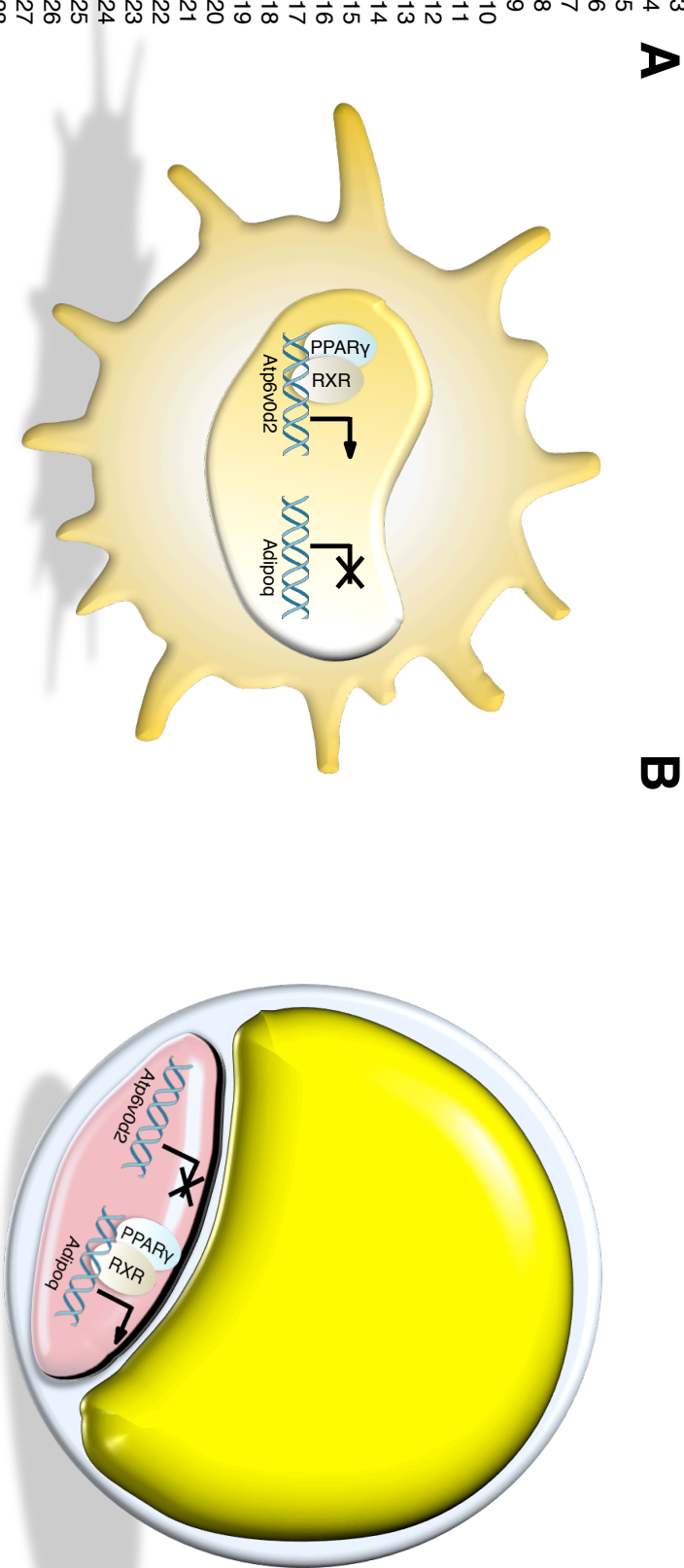
3D imaging.

Fluorescent microscopy or contrast imaging technics sections can be processed by computational specific software's. The data obtained from this approach can be used

to draw models from cells to whole organism behavior after a stimulus or during developmental stage.

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Figure 1



A

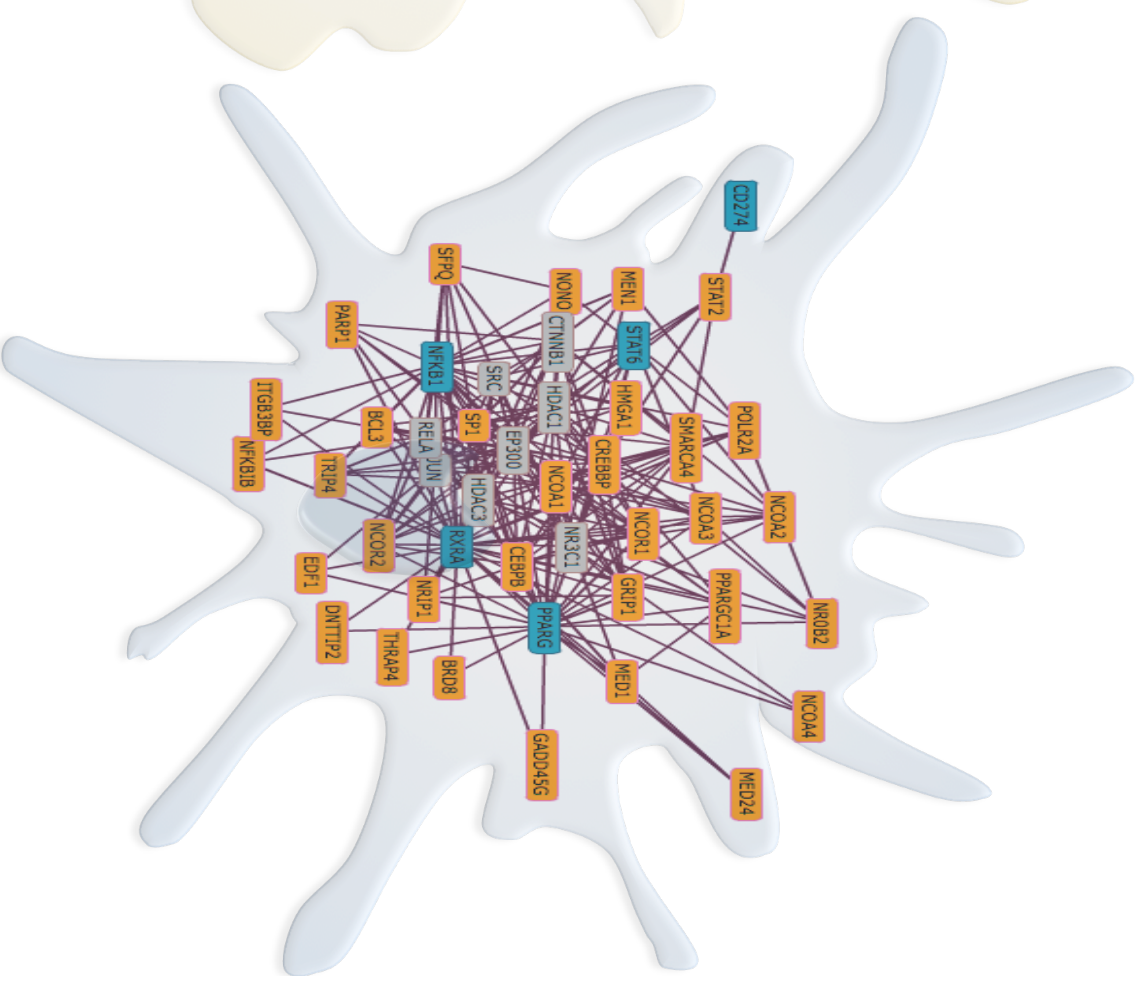
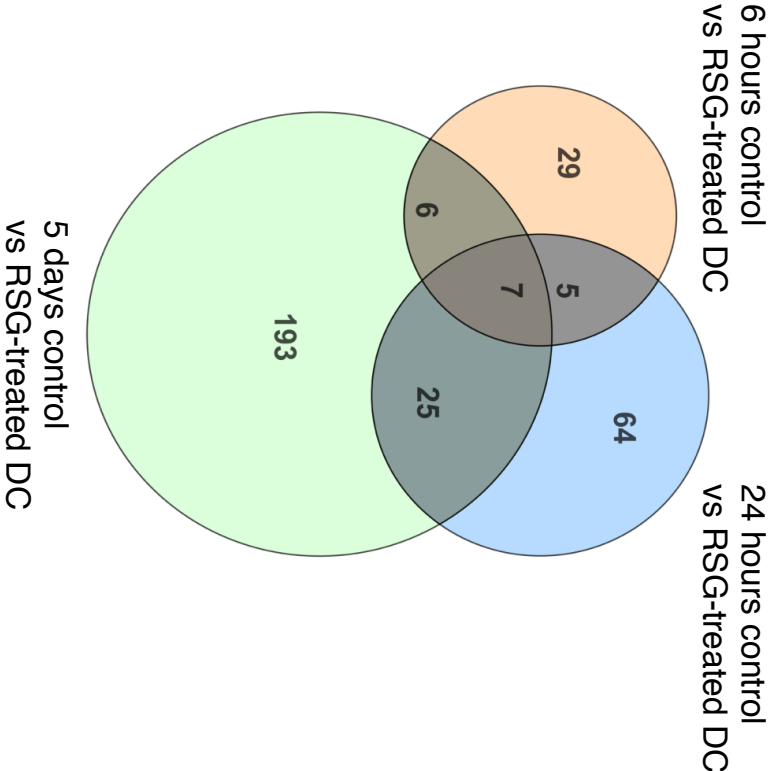


Figure 3

A



B

6 hours control vs RSG treated DC	
GO Term	p-value
long-chain fatty acid transport	1.5770855E-6
fatty acid transport	3.510332E-6
regulation of phagocytosis, engulfment	1.5830125E-5
positive regulation of phagocytosis, engulfment	1.5830125E-5
lipid storage	2.2889573E-5

24 hours control vs RSG treated DC	
GO Term	p-value
immune system process	5.3502758E-12
extracellular region part	2.0419205E-11
immune response	3.9918048E-11
extracellular region	7.636777E-11
cytokine activity	9.974949E-10

5 days control vs RSG treated DC	
GO Term	p-value
lipid metabolic process	9.655801E-10
cellular lipid metabolic process	5.0148725E-9
lipid biosynthetic process	2.625176E-7
membrane	1.1664425E-6
triglyceride metabolic process	7.4364416E-6