Published in final edited form as:
Trends Pharmacol Sci. 2011 September ; 32(9): 543–550. doi:10.1016/j.tips.2011.04.004.

Targeting GPR120 and other fatty acid sensing GPCRs ameliorates insulin resistance and inflammatory diseases

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Abstract

The last decade has seen great progress in the understanding of the molecular pharmacology, physiological function and therapeutic potential of the G protein-coupled receptors. Free Fatty acids (FFAs) have been demonstrated to act as ligands of several GPCRs including GPR40, GPR43, GPR84, GPR119 and GPR120. We have recently shown that GPR120 acts as a physiological receptor of ω3 fatty acids in macrophages and adipocytes, which mediate potent anti-inflammatory and insulin sensitizing effects. The important role GPR120 plays in the control of inflammation raises the possibility that targeting this receptor could have therapeutic potential in many inflammatory diseases including obesity and type 2 diabetes. In this review, we discuss lipid-sensing GPCRs and highlight potential outcomes of targeting such receptors in ameliorating disease.

Lipid sensing GPCRs as therapeutic targets

G-protein-coupled receptors (GPCRs) comprise a family of cell-surface receptors that respond to various extracellular stimuli such as light, odorants, neurotransmitters and hormones, and trigger a cascade of intracellular signaling. There are approximately 850 predicted human GPCRs [1] that have specific cell type or tissue-specific expression and are involved in various physiological and clinical processes [2]. The importance of identification and characterization of GPCRs is underscored by the fact that ~ 30% of all prescription drugs target GPCRs [3]. Because these drugs modulate approximately half of the well characterized GPCRs, it is likely that many more GPCRs remain to be explored as potential drug targets. GPCRs in humans and mice show striking orthology, and the fact that highly related receptors are evolutionarily conserved, suggests that these receptors are not functionally redundant[2]. Because human diseases involving GPCR mutations are extremely rare, occurring in less than 1 per 1000 people, they also provide a stable therapeutic target [4].

Free fatty acids (FFAs) can act as ligands of several GPCRs, including GPR119, GPR84, GPR120, GPR40 (FFAR1), GPR43 (FFAR2) and GPR41 (FFAR3) (Table 2). Fatty acids are categorized by the length of their aliphatic tails; short-chain fatty acids have less than 6 carbons, medium-chain fatty acids have 6-12 carbons and long-chain fatty acids have 12 or more carbons. Fatty acids can act as signaling molecules that modulate receptor signaling and gene expression [5]. GPR120 and GPR40 are activated by medium- and long-chain FFAs,

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and GPR119 is activated by long-chain fatty acids. GPR84 is activated by medium-chain fatty acids, whereas GPR43 and GPR41 are activated by short-chain FFAs [6].

In obesity and type 2 diabetes, elevated levels of plasma FFAs are observed, resulting in lipid accumulation and insulin resistance in target tissues [7]. FFAs exert divergent effects on insulin secretion from beta cells. Acute exposure to FFAs stimulates insulin secretion, whereas chronic exposure impairs insulin secretion [7]. The dual and opposing effects of FFAs on insulin secretion raise the possibility that FFAs contribute to both hyper- and hypo-insulinemia during the development of type 2 diabetes. Thus, GPCRs that recognize fatty acids are of particular interest in the treatment of type 2 diabetes [8].

Chronic low grade metabolic inflammation, referred to as 'metaflammation' [9], has been established in the pathogenesis for obesity, insulin resistance and type 2 diabetes. The contribution of inflammation to insulin resistance has been extensively studied, and immunological changes occurring in adipose tissue, liver, brain, islets, the vasculature and circulating leukocytes, with concomitant changes in cytokines and chemokines are important components of the etiology of insulin resistance and type 2 diabetes [9, 10]. Although recent studies have identified the presence of different cells of the adaptive immune system, such as T cells and mast cells in adipose tissue, macrophages are the most abundant leukocyte population and are generally considered as the effector cell contributing to inflammation-mediated insulin resistance [10, 11]. In addition, macrophage-mediated inflammation has been associated with many other diseases such as rheumatoid arthritis, cancer, inflammatory bowel disease, cardiovascular disease [1213], psoriasis [14], multiple sclerosis [15] and periodontitis [16]. A very interesting recent study showed that macrophages might mediate metastasis in breast cancer [17]. Macrophages can express a wide variety of GPCRs that modulate physiological processes such as inflammation and immunity. Furthermore, the expression of GPCRs differ in pro- and anti-inflammatory macrophages [18]. The identification of endogenous and synthetic ligands to target inflammatory disease is therefore of great importance, and FFA-sensing GPCRs expressed in macrophages and insulin target tissues have emerged as therapeutic targets.

Medium- and long-chain fatty acid receptors

GPR120

We have shown that GPR120 is a physiological receptor for ω3 fatty acids in macrophages and adipocytes, mediating potent anti-inflammatory and insulin sensitizing effects[19]. ω3 fatty acids are part of a series of essential polyunsaturated fatty acid family that cannot be synthesized *de novo* within the human body, but are vital for normal metabolism [20]. GPR120 is a member of the rhodopsin-like family of GPCRs and is highly conserved across many species [21]. Long-chain fatty acids, in particular palmitoleic acid (PA), and the ω3 fatty acids, α-linolenic acid (ALA), docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are established activators of GPR120 [19, 22]. Some studies have shown that stimulation of GPR120 by these fatty acids increases glucagon like peptide-1 (GLP-1) secretion both *in vitro* and *in vivo*, and consequently increases circulating insulin levels. Stimulation of GPR120 by ALA and PA increases cholecystokinin secretion *in vivo* and in mouse intestinal enteroendocrine cells [23]. Upon extensive tissue expression analyses, we found that GPR120 is expressed in macrophages and mature adipocytes and that the expression increases in pro-inflammatory macrophages. We demonstrated that DHA abolishes LPS-mediated phosphorylation and activation of IκB kinase (IKK) and c-Jun N-terminal kinase (JNK) in macrophages; upon GPR120 knockdown, DHA has no effect. Activation of GPR120 by DHA recruits β-arrestin 2 (β-arr 2) to the cytosolic putative binding sites on GPR120, and the GPR120- β-arr 2 complex internalizes. Pro-inflammatory cascades are broadly mediated by the tumor necrosis factor-alpha (TNF-α) and toll-like receptor 4 (TLR4) receptors. Both

of these pathways converge at the step of TGF-beta activated kinase 1 (TAK1) interacting with TGF-beta activated kinase 1 binding protein 1 (TAB1) and mediate downstream inflammatory effects via activation of NF-κB and JNK. The internalized GPR120-β-arr 2 complex interacts with TAB1, thereby inhibiting the TAB1 interaction with TAK1 and in effect, inhibiting the downstream pro-inflammatory pathways.

GPR120 activation in primary adipose tissue and 3T3-L1 adipocytes led to an increase in glucose transport and translocation of GLUT4 to the plasma membrane, and this effect was abolished upon GPR120- and Gαq/11- KO. Knockdown of GLUT4 using siRNA abolished the stimulatory effects of GPR120 agonists, whereas knockdown of β-arrestin 1 and 2 was without effect. GPR120 activation in primary adipose tissue and 3T3-L1 adipocytes led to an increase in glucose transport and translocation of GLUT4 to the plasma membrane, and this effect was abolished upon GPR120- and Gαq/11- KO. Knockdown of GLUT4 using siRNA abolished the stimulatory effects of GPR120 agonists, whereas knockdown of β-arrestin 1 and 2 was without effect.

Interestingly, we demonstrated that the anti-inflammatory effects of ω3 fatty acids mediated by GPR120 are exclusively dependent on β-arr2, but independent of Gαq/11, regardless of the fact that GPR120 can be a Gαq/11-coupled receptor in other contexts [19]. These mechanistic differences in macrophage and adipocytes is consistent with the concept of ‘functionally selective’, or ‘biased agonism’, which postulates that some agonists can cause unique agonist-specific active state conformations of receptors, which can result in a differential effector response in various cell types. In such scenarios, the efficacy of a drug is affected by the cell type, because cells vary in the relative stoichiometry of intracellular signaling components [24]. The mechanism by which activated GPR120 inhibits inflammation in the macrophage is summarized in Figure 1. Because activated GPR120 inhibits TLR2, TLR4 and TNFR-mediated inflammation, it is possible that a wide range of diseases mediated by one, or any of these pathways, can be targeted via GPR120 agonists.

We performed *in vivo* studies with GPR120 knockout (KO) mice and wild-type (WT) littermates on a standard chow diet, a 60% high-fat diet (HFD) and a 60% high-fat diet containing ω3 FAs (ω3 diet). On standard chow, the GPR120 KO animals were more insulin resistant compared to WT controls. Both WT and GPR120 KO animals were equally insulin resistant upon HFD feeding. However, upon ω3 supplementation, the WT animals showed significantly improved glucose tolerance compared to both WT and KO animals on HFD alone (Table 1). ω3 supplementation was without effect in the GPR120 KO animals. Because the HFD typically contains negligible amount of ω3 fatty acids, and standard chow contains more ω3 compared to HFD, we hypothesized that the phenotype of these animals was directly correlated to the amount of ω3 fatty acids obtained from diet, and validated this using lipidomic analyses. We determined that circulating concentrations of ω3 FAs were much lower in mice on a HFD compared to chow diets, and supplementing the HFD with ω3 FAs led to an increase in plasma ω3 FA levels. To confirm the contribution of macrophages to the *in vivo* phenotype, we performed bone marrow transplants to obtain functional macrophage-specific GPR120 knockout mice, and found that ω3 supplementation was only effective in the WT, but not macrophage-specific GPR120 knockout mice [19].

Our study demonstrates that GPR120 plays an important role in the control of inflammation, and raises the possibility that many inflammatory disease states (including obesity, diabetes, cancer and cardiovascular disease) could potentially be attenuated by ω3 FA supplementation. The current rise in inflammatory diseases underscores the importance of exploring effective and safe therapeutic strategies. The anti-inflammatory potential of ω3 FAs has been well investigated and shown to have therapeutic potential in numerous diseases [12]. There have been multiple clinical trials assessing the benefits of dietary

supplementation with fish oils in the prevention and/or treatment of several inflammatory and autoimmune diseases including, rheumatoid arthritis[13], periodontitis [16], bronchial asthma [25], cardiovascular disease [26], cancer [27] and neuroprotection [28]. In general the effects observed in these clinical trials vary widely from subtle to moderate, and the most beneficial dose in humans is still under investigation [29]. However, in some studies ω3 supplementation did not improve the inflammatory status, for example in patients treated with 3.4 g/d of EPA +DHA the serum triglycerides were significantly lowered but no improvement in inflammatory status was observed after 8 weeks of treatment [30]. The concept of achieving effective functional dosing *in vivo* is discussed in a recent review in the context of inflammatory disease [31] . Schall *et al.,* discuss that time, target and dose are the three crucial factors that act synergistically to determining effective therapy. Before ruling out the potentially therapeutic beneficial effects of ω3 supplementation in studies where no effect on inflammatory status is observed, further attention must be given to the dose used, the duration of treatment while also controlling for omega 6 and saturated fatty acid intake while administering omega 3 supplements.

Beneficial effects of ω3 diets in inflammatory disease might involve improvements in macrophage function related to reversal of defective macrophage phagocytosis of apoptotic cells (efferocytosis) [32]. In obesity, type 2 diabetes and atherosclerosis, elevated levels of saturated FAs and/or decreased levels of ω3 FAs contribute to decreased efferoctyosis. There is an increased content of saturated FAs and decreased DHA and eicosapentaenoic acid (EPA) compared to controls, in membrane lipid composition of ob/ob and ob/ob;Ldlr(-/-) macrophages. Defective macrophage efferocytosis in ob/ob macrophages can be reversed by treatment with EPA or by feeding ob/ob mice a ω3-rich diet [32], demonstrating the beneficial effects of ω3 supplements in genetic models of obesity.

The importance, and need for ω3 supplementation is highlighted by the fact, that the dietary intake ratio of ω6:ω3 fatty acids in humans has changed dramatically from 1:1 in early humans, to 10:1 in the United States as of 2002. This is due to a combined effect of decreased intake of ω3, and increased intake of ω6 [26]. The American Heart Association (AHA) recommends ω3 FA intake as prevention agents against cardiovascular disease [26], and ω3 fatty acids are also prescribed for patients with hypertriglyceridemia [33]. Interestingly, GPR120 is expressed in the taste buds, and GPR120 KO mice show a diminished preference for linoleic acid and oleic acid, and diminished taste nerve responses to several fatty acids [34]. These results show that GPR120 mediates the taste of fatty acids[34]. Further studies are required to elucidate whether GPR120 expression in taste buds can cause preferential disposition towards a healthy and beneficial ω3 diet.

Type 2 diabetic subjects often require a combination of dietary, pharmacological and lifestyle interventions. One study in a diet-induced obese mouse model demonstrated that low-dose thiazolidinedione treatment in combination with dietary ω3 supplementation is a very viable and attractive option, because it can help offset the side effects of thiazolidinedione (TZD) treatment alone, such as edema, weight gain, bone loss and possible risk of heart failure [35]. Clinically, ω3 FAs have been successfully used in combination with simvastatin in the treatment of various lipid disorders such as increased Low-density lipoprotein, triglycerides, non-high-density lipoprotein cholesterol and lipoprotein particle size. In subjects with persistent hypertriglyceridemia, prescription ω3-acid ethyl ester (P-OM3; Lovaza) 4g/day plus simvastatin 40mg/day and dietary counseling improved non-HDL-C and other lipid and lipoprotein parameters to a greater extent than simvastatin alone [36].

Clinical studies of ω3 fatty acids

Clinical studies have established that dietary intake of ω3 fatty acids should be around 4g/day for treating very high triglyceride levels (≥ 500 mg/dL) [37]. Low-dose supplementation with EPADHA (400mg/day) or ALA (2 g/day) did not significantly reduce the rate of major cardiovascular events among patients who had a myocardial infarction, and who were receiving state-of-theart antihypertensive, antithrombotic and lipid-modifying therapy [38]. The dose of ω3 fatty acids used in clinical trials varies widely from approximately 400 mg - 16.2 g per day, and the most beneficial dose in humans is still under investigation [27, 29, 30, 39]. The most commonly reported adverse effect of ω3 fish oil supplements is a fishy aftertaste and eructations, but several others have been reported, including nausea and gastric bleeding [12, 26, 40, 41]. Among the more serious effects, one study suggested that omega 3 supplements may result in a slightly higher risk of hemorrhagic stroke [42]. Consumption of high levels of fish oil can also result in hypervitaminosis, a condition associated with high levels of Vitamins -A and –D, that are usually added to the fish oils [43].

Clinically, low doses of ω3 FAs are largely ineffective, even in the context of combination therapy. Should fish oils prove impractical as a therapeutic agent, the identification of the GPR120 receptor suggests that synthetic DHA/EPA mimetics could be developed that might provide the same and potentially greater anti-inflammatory effects. Synthetic agonists have certainly been more potent than endogenous ligands, as demonstrated by synthetic agonists of liver X receptors (LXRs) [44] and farnesoid X receptor (FXRs) [45]. A recent report suggested potential synthetic agonists of GPR120 based on molecular modeling [46], that could be further explored to obtain clinical benefits.

GPR40 (FFAR1)

Free fatty acid receptor 1 (FFAR1) or GPR40, plays an important part in the mechanisms that link obesity and type 2 diabetes. GPR40 mediates both acute and chronic effects of FFAs on insulin secretion, and GPR40 signaling is linked to impaired glucose homeostasis. GPR40 is preferentially expressed in pancreatic beta cells, where it plays a role in the FFA enhancement of glucose-stimulated insulin secretion [47-50]. GPR40 is also expressed in endocrine cells of the gastrointestinal tract, including cells that express incretin hormones such as GLP-1 and glucose-dependent insulinotropic polypeptide (GIP). In addition, GPR40 mediates FFA-stimulated incretin secretion [51].

Loss of GPR40 protects mice from obesity-induced hyperinsulinemia, hepatic steatosis, hypertriglyceridemia, increased hepatic glucose output, hyperglycemia, and glucose intolerance, and overexpression in mice leads to beta cell dysfunction, hyperinsulinemia and diabetes [52]. Loss of GPR40 function through small interfering RNA (siRNA) [47, 53] or pharmacological inhibition [54] suppresses FFA-stimulated potentiation of glucose-stimulated insulin secretion. However, these findings are controversial, as more recently, Kebede *et. al.* have shown that GPR40 KO mice develop fasting hyperglycemia and they became as obese, glucose intolerant, and insulin resistant as their WT littermates given 8 weeks of HFD feeding. [55], Table 1. Furthermore, some studies report that deletion of GPR40 could not improve dysfunction of pancreatic islets and insulin resistance caused by a high fat diet *in vivo* [56, 57] and over-expression of GPR40 in islets improves glucose tolerance [58].

Due to its biological activity and tissue distribution, GPR40 is an attractive drug target for type 2 diabetes, but a debate still exists as to whether an agonist or antagonist may be therapeutic. A GPR40 agonist, GW9508, activates both GPR40 and GPR120 and stimulates glucose-stimulated insulin secretion in insulin-secreting MIN6 cells [54]. TZDs including rosiglitazone and MEDICA16 also activate GPR40 [59, 60]. A recent study showed a small

molecule antagonist of GPR40, DC260126, decreased serum insulin levels and improved insulin tolerance, but not glucose tolerance in Zucker rats [61].

GPR84

GPR84 functions as a receptor for medium-chain FFAs and is highly expressed in leukocytes [62]. Activation of GPR84 in monocytes/macrophages amplifies LPS-stimulated IL-12 p40 production [62] and expression of GPR84 is markedly induced in macrophages (including glial cells) under inflammatory conditions [63]. GPR84 KO mice have normal T cell and B cell proliferation and increased Th2 cytokine production [64], (Table 1). The leukocyte-specific expression of GPR84 suggests it may be involved in linking fatty acid metabolism to immunological regulation [62]. Further studies of KO animals upon high fat diet feeding are required to better elucidate the mechanisms by which this receptor modulates glucose homeostasis.

GPR119

GPR119 is a Gαs-coupled receptor that is expressed in the pancreas, ileum and colon and binds long-chain fatty acids including oleoylethanolamide (OEA), lysophosphatidylcholine (LPC), lipid amides, and retinoic acid, with OEA being the most potent, and efficacious. Some controversy exists about retinoic acid and LPC being bona fide ligands [65]. Activation of GPR119 by synthetic agonists results in an increase the release of insulin and GLP1, and significantly improves glucose tolerance [66]. This highly desirable combination of stimulating both insulin and incretin release has attracted the interest of numerous pharmaceutical companies, and clinical trials are ongoing to determine the efficacy of GPR119 agonists in human patients.

GPR119 KO mice on normal chow show normal plasma glucose and lipids, but have lower body weight and lower post-prandial levels of active glucagon-like peptide 1 (GLP-1) [67], (Table 1). Furthermore, nutrient-stimulated GLP-1 release is attenuated in *GPR119*$^{-/-}$ mice, suggesting that GPR119 plays a role in physiological regulation of GLP-1 secretion. On a high fat diet, GPR119 KO mice gain weight similarly to WT littermates, and glucose and insulin tolerance tests did not reveal a genotypic difference, suggesting that GPR119 is not essential for the maintenance of glucose homeostasis.

Short-chain fatty acid receptors

GPR43 and GPR41 are both activated by short-chain FFAs such as formate, acetate, propionate, butyrate, and pentanoate [60, 68, 69]. GPR43 and GPR41 differ in their specificity for ligands with different length carbon chains (Table 2). GPR41 is activated equally by propionate, butyrate, and pentanoate, whereas GPR43 prefers propionate to other short-chain FFAs [68, 69].

GPR43 (FFAR2)

Free Fatty Acid Receptor 2 or GPR43 is activated by short-chain fatty acids and is involved in the regulation of fatty-acid and glucose homeostasis in adipose tissue and the intestines; thus it has potential therapeutic relevance in the treatment of type 2 diabetes, insulin resistance and obesity [70]. GPR43 expression is induced during adipocyte differentiation, and increased by high fat feeding in rodents, suggesting it may also affect adipocyte function [71-73]. Adipocytes treated with natural ligands, acetate and propionate, exhibit a reduction in lipolytic activity that is not observed in GPR43 KO mice [71, 73]. Furthermore, in humans, acetate reduces plasma FFA levels [74, 75]. Therefore, GPR43 plays a role in regulating plasma lipid profiles and aspects of the metabolic syndrome, a term that refers to

a cluster of related abnormalities including insulin resistance, dyslipidemia and hypertension. [71].

GPR43 is highly enriched in immune cells, particularly in the polymorphonuclear cells [60, 68, 69], and to a lesser extent in bone marrow, spleen and fetal liver [68]. Expression is induced by leukocyte differentiation into monocytes and neutrophils, suggesting GPR43 has a role in the activation process of leukocytes [76]. GPR43 is expressed in enteroendocrine cells expressing peptide Y (PYY) [71, 77] and in mucosa and in mucosal mast cells that contain 5-hydroxytryptamine (5-HT) [78]. Short chain FFAs stimulate release of PYY [79] and 5-HT [80] from the ileum and colon.

GPR43 is also observed in rat and human colon wall and functions as a tumor suppressor by mediating SCFA-induced cell proliferation inhibition and apoptotic cell death in colon cancer [81].

GPR43 KO mice on high-fat diet results in decreased body mass and increased lean mass compared to WT littermates, (Table 1). The KO animals have improved glucose tolerance, increased energy expenditure, increased core body temperature, increased food intake, decreased triglyceride levels in the liver, decreased plasma cholesterol, decrease in lipid in brown adipose tissue, decreased crown like structures in the fat [82]. Targeting GPR43 by dietary manipulation seems attractive at first glance, however studies with the GPR43 agonist, butyrate have raised concerns as a possible associated link with the development of colon cancer. The contribution of butyrate resulting from highly fermentable dietary fibers, to colon cancer is currently controversial with conflict between *in vitro* and *in vivo* studies, referred to as the “butyrate paradox” [83].

FFAR3 (GPR41)

Free fatty acid receptor 3 (FFAR3) or GPR41 is expressed abundantly in adipose tissue [68] and has been implicated in leptin production by stimulation of short chain FFAs in adipocytes [84]. Leptin production is increased by the over-expression of exogenous GPR41 and abolished by the knock down of GPR41 expression with siRNA [84]. Acute oral administration of propionate increases circulating leptin levels in mice, which suggests that the effects of propionate are mediated partially via GPR41 *in vivo* but other short chain FFARs activated by propionate, (i.e. FFAR2) may also be involved in this response [68, 84]. GPR41 KO mice have reduced PYY expression, increased intestinal transit rate and reduced extraction of energy from short chain FFAs that are produced by the microbial fermentation of indigestible dietary polysaccharides (Table 1). These results suggest that GPR41 regulates host energy balance through mechanisms that are dependent upon the gut microbiota [85].

The fact that endogenous agonists of both GPR41 and GPR43 are by-products generated by fermentation of dietary fiber by the gut microbes suggests the importance and relevance of the biome in inflammatory diseases. The gut hosts ~1×10^{14} bacteria from 500–1,000 different species, that are ten times greater than the total number of cells in humans. Research in the last few years has revealed that gut microbes shape and influence the intestinal immune system. The current view that the impact of the intestinal microflora on host immunity stops at the gut [86] is being challenged with new studies identifying an intrinsic role of these microbes contributing to metabolic disease [87]. Tables 1 and 2 summarize the expression, ligands and physiological roles of the GPCRs discussed in this review.

Concluding remarks

Fatty acids are not only essential nutrients, but play important roles as endocrine regulators of lipid and carbohydrate metabolism through the activation of their cognate GPCRs. FFARs play significant roles in nutritional regulation by sensing both - and short-chain fatty acids. The deorphanization of more GPCRs is likely to identify additional receptors of endogenous signaling molecules, including FFAs. Our recent study established GPR120 as a physiological receptor of ω3 fatty acids in macrophage and adipocytes that mediates potent anti-inflammatory and insulin sensitizing effects. Targeting fatty acid sensing GPCRs to ameliorate inflammatory disease is an exciting and growing area, and many clinical trials are currently underway to explore the beneficial anti-inflammatory therapeutic effects of ω3 fatty acids in a broad spectrum of disease. Hippocrates in his wisdom clearly nailed it when he said almost 2500 years ago, “Let food be your medicine and let medicine be your food. Only nature heals, provided it is given the opportunity”[88]. In the context of the global diabetes and inflammatory disease epidemic, we should have heeded his words sooner.

Acknowledgments

We thank Dr Min Lu and Dr William S Lagakos for critically reading this manuscript.

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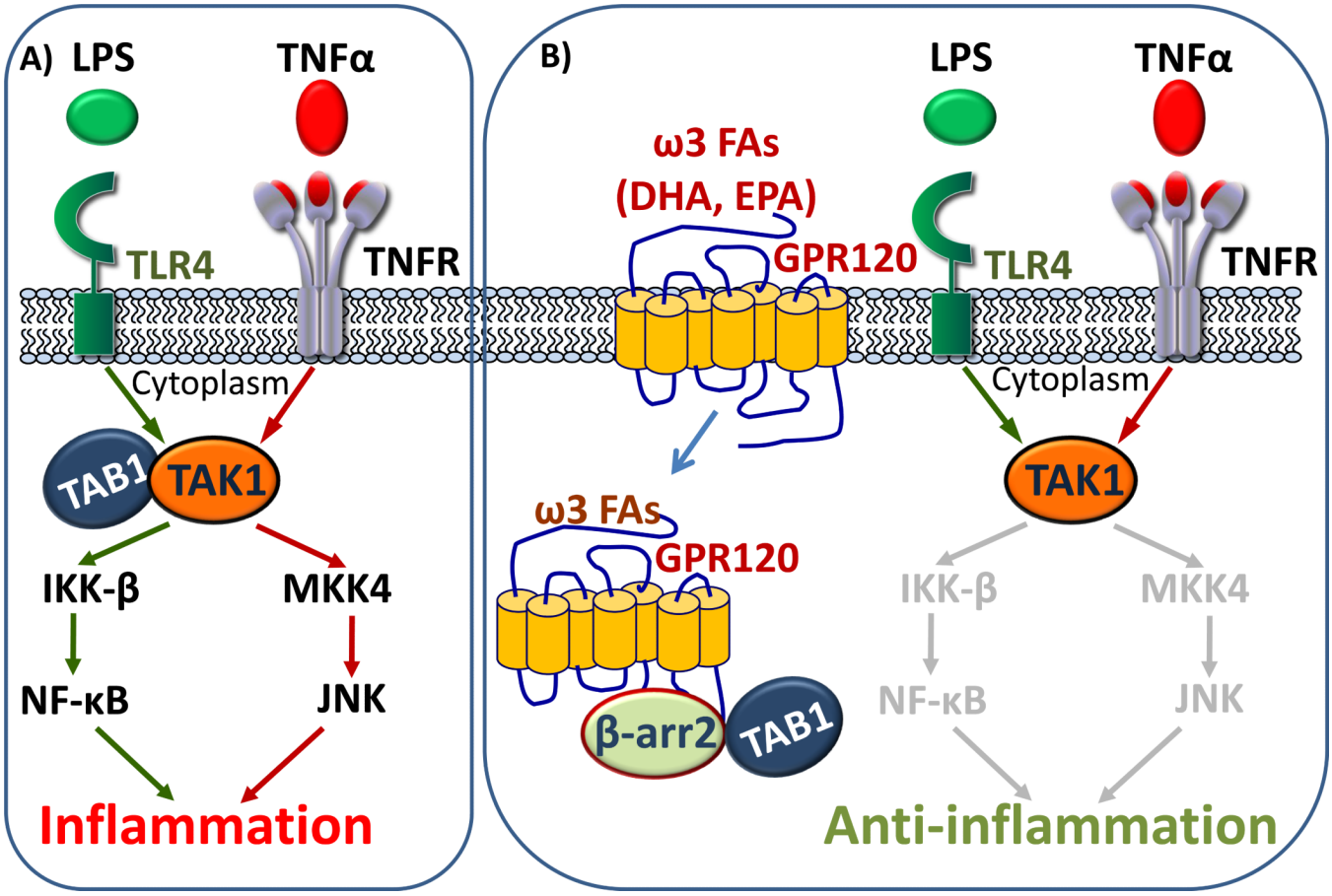

Figure 1. Mechanism of anti-inflammation upon GPR120 activation by omega 3 fatty acids in macrophage

A) Activated TLR4 and TNFR by LPS and TNFα respectively, converge on cytoplasmic association of TAK1 with TAB1, mediating pro-inflammatory cascades by activating NF-B and JNK1. B) Activation of GPR120 by ω3 FAs internalizes GPR120 which binds to β-arrestin 2 and sequesters TAB1, inhibiting inflammation.

Table 1

Metabolic phenotype of free fatty acid receptor knock out mice.

GPCR	HF diet phenotype	reference
GPR120KO	Normal chow: GPR120 KO animals were more insulin resistant compared with WT controls. High fat diet: Both WT and GPR120 KO animals were equally insulin resistant. However, upon ω3 supplementation, the WT animals had significantly improved glucose tolerance compared to both WT and KO animals on HFD. ω3 supplementation was without effect in the GPR120 KO animals.	19
GPR119KO	Normal chow: GPR119 KO mice show normal plasma glucose and lipids, but have lower body weight and lower post-prandial levels of active glucagon-like peptide 1 (GLP-1)[67]. Nutrient-stimulated GLP-1 release is attenuated in GPR119$^{-/-}$ mice. High fat diet: GPR119KO mice gain weight similarly to wild type littermates and show similar results during glucose and insulin tolerance tests.	67
GPR40KO	High fat diet: for 8 wks. Loss of GPR40 protects mice from obesity-induced hyperinsulinemia, hepatic steatosis, hypertriglyceridemia, increased hepatic glucose output, hyperglycemia, and glucose intolerance.	52
	High fat diet: for 11 wks: GPR40KO mice develop fasting hyperglycemia, become obese and glucose intolerant and insulin resistant with similar liver steatosis as WT littermates.	55
GPR84KO	Normal chow: Normal T- and B-cell proliferation in KO mice, increased Th2 production in KO mice, regulates early IL-4 gene expression in activated T cells.	64
GPR43KO	Normal chow: GPR43KO mice are phenotypically normal. High fat diet: GPR43KO show decreased body mass, increased lean mass, Improved glucose tolerance, increased energy expenditure, increased core body temperature, increased food intake, decreased triglycerides in liver, decreased plasma cholesterol, decreased lipid in brown adipose tissue and decreased crown-like structures in fat.	73
GPR41KO	GPR41KO mice have reduced PYY expression, increased intestinal transit rate and reduced extraction of energy from short chain FFAs that are produced by the microbial fermentation of indigestible dietary polysaccharides. These results suggest that GPR41 regulates host energy balance through mechanisms that are dependent upon the gut microbiota.	85

Table 2

FFA sensing GPCRs and their cognate ligands

GPCR	Ligands	Synthetic agonists
GPR40	Long chain fatty acids (C12-C16)	TAK-875 (*ACS Med. Chem. Lett.,* 2010, *1* (6), pp 290–294), GW9508 [51], MEDICA16 (relatively selective for GPR40), Troglitazone and Rosiglitazone[89].
GPR41	Short chain fatty acids (C3-C5) formate, acetate, propionate, butyrate and pentanonate[89]	
GPR43	Short chain fatty acids (C2-C3) formate, acetate, preferentially propionate, butyrate and pentanonate[89]	ESN-280 and ESN-282[70].
GPR120	Long chain fatty acids (C14-1C8) Omega 3 fatty acids, EPA, DHA, palmitoleic acid, α-linolenic acid (ALA)	NCG compound, grifolic acid (partial synthetic agonist) and MEDICA16[46].
GPR119	Oleoylethanolamine and *N*-oleoyldopamine	Several, such as AR231453, WO07120702, JP04269468, JP04269469, WO06043490[65]
GPR84	Medium chain fatty acids, Capric acid (C10:0), undecanoic acid (C11:0), and lauric acid (C12:0)[62]	