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Mini Review

Emerging roles of Lipasin as a critical lipid regulator

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ABSTRACT

Patients with metabolic syndrome are at high risk for developing atherosclerotic cardiovascular diseases and diabetes. In addition to total cholesterol, LDL-C and HDL-C, elevated plasma triglycerides (TG) are increasingly recognized as an independent risk factor for cardiovascular diseases. Recently 3 groups independently reported the identification and characterization of a novel blood lipid regulator, Lipasin/RIFL/ANGPTL8, which here is referred to as Lipasin for its lipoprotein lipase inhibition effect and for being a circulating factor denoted by 'in'. Being highly enriched in the liver, Lipasin is a hepatocyte-derived circulating factor that regulates plasma TG levels. Lipasin is nutritionally regulated, as its mRNA levels in liver and fat as well as its protein level in serum are reduced by fasting. Mice deficient for Lipasin have lower serum TG levels; conversely, its adenovirus-mediated overexpression increases serum TG levels, in part, through promoting ANGPTL3 cleavage, releasing its N-terminal domain that inhibits lipoprotein lipase. Lipasin sequence variations are associated with LDL-C and HDL-C concentrations in humans. Being lipogenic, Lipasin is highly induced during adipogenesis. Levels of Lipasin and ANGPTL4 show opposite changes in response to fasting or cold environment. Lipasin, a novel but atypical ANGPTL family member, is emerging as a critical lipid regulator and a potential drug target.

1. Introduction

The prevalence of metabolic syndrome, a common metabolic disorder including glucose intolerance and dyslipidemia, has increased dramatically in the past two decades [1], largely due to the global obesity epidemic [2]. Patients with metabolic syndrome are at high risk of developing atherosclerotic cardiovascular diseases and diabetes, posing a major public health issue. Of note, in addition to total cholesterol, LDL-C and HDL-C [3], increasing epidemiological evidence indicates that plasma triglyceride (TG) level is an independent risk factor for cardiovascular diseases [4]. Plasma TGs are determined by the balance between their production and clearance, where the former involves chylomicrons (CMs) that are predominately synthesized in the intestine in the postprandial state and VLDL that is synthesized in the liver in the fasted state, and the latter involves lipoprotein lipase (LPL) mediated clearance in peripheral tissues. Patients with hypertriglyceridemia have increased plasma levels of remnants from CMs and VLDL, which penetrate the arterial endothelium, are preferentially trapped within subendothelial space [5,6], and can lead to the development of atherosclerosis and coronary heart diseases [7]. Lowering TG levels pharmacologically therefore may provide an additional strategy for preventing cardiovascular diseases.

Recently 3 groups almost simultaneously reported the identification and characterization of a novel blood lipid regulator, called Lipasin [8,9], RIFL [10] or ANGPTL8 [11]. The official symbols for this gene are Gm6484 (mouse) and C19orf80 (human). We here refer to this gene as Lipasin, which is a novel but atypical member of the angiopoietin-like protein family. Here we review the current knowledge about Lipasin regarding its expression, regulation, function and mechanisms of action and discuss its potential roles in human physiology and pathology.

2. The angiopoietin-like protein family (ANGPTLs)

The ANGPTL protein family contains 7 typical members, ANGPTL1–7, encoded by 7 different genes [12]. ANGPTLs have structural homology to that of angiopoietins, hence the name angiopoietin like proteins. ANGPTLs are characterized by the presence of a coiled-coil domain at the N-terminus, a fibrinogen like domain at the C-terminus and a signal peptide that is typical for secreted proteins (Fig. 1). Indeed, ANGPTL 2, 3, 4 and 5 have been detected in the systemic circulation, suggesting that these ANGPTLs may function in an endocrine manner [12]. Although angiopoietins bind to TIE2 and TIE1 receptor tyrosine kinases, the signaling that regulates vascular remodeling, none of the 7 ANGPTLs binds to these receptors, suggesting that the physiological function of ANGPTLs and angiopoietins are different (Table 1). Indeed, the roles of ANGPTL3 and ANGPTL4 in lipid regulation have received much

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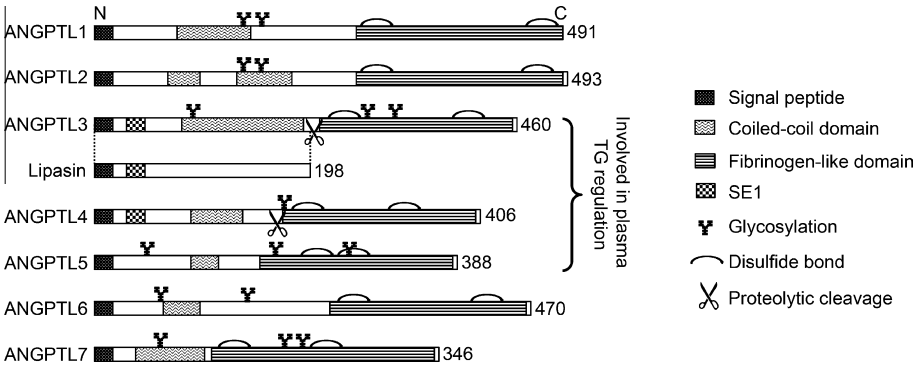

Fig. 1. Domains and protein modifications of Lipasin and ANGPTLs. Figure not drawn to scale. Dotted lines denote homologous regions. SE1, a region mediating lipoprotein lipase binding; TG, triglyceride.

Table 1
Comparison among Lipasin, ANGPTLs and Angiopoietins.

	Lipasin[a]	ANGPTLs[b]	Angiopoietins
Signal peptide	√	√	√
Secreted	√	√	√
Coiled-coil domain	––[c]	√	√
Fibrinogen-like domain	––[c]	√	√
Glycosylation	––[c]	√	√
Disulfide bond	––[c]	√	√
Bind to TIE2	NR	––	√

√, present; ––, absent; NR, not reported.
[a] Symbols for Lipasin are Gm6484 (mouse, NP_001074409) and C19orf80 (human, NP_061157), and other names include TD26, PRO1185, PVPA599, RIFL and ANGPTL8.
[b] Human ANGPTL members include ANGPTL1–7, with IDs being NP_004664, NP_036230, NP_055310, NP_647475, NP_835228, NP_114123 and NP_066969, respectively.
[c] Predicted. Source, Uniprot.

attention in the past decade, and a large body of evidence has shown that both ANGPTL3 and ANGPTL4 are involved in lipid storage and breakdown.

3. ANGPTL3

The role of ANGPTL3 in lipid metabolism was discovered by Koshi and coworkers by positional cloning of recessively inherited mutations in the mutant KK/San mice, which exhibit extremely low serum TG levels, and they found a 4-bp insert in exon 6 of the ANGPTL3 gene, causing a frame shift mutation [13]. They further showed that adenovirus-mediated overexpression of ANGPTL3 or injection of its recombinant protein increase plasma TG levels in both KK/San and wild-type C57BL/6 mice [13]. Conversely, deletion of ANGPTL3 in mice lowers serum TG and cholesterol levels [14].

Subsequent studies showed that ANGPTL3 increases serum TG by inhibiting LPL activity. Indeed, low plasma TG levels in the KK/San mice were mainly due to increased LPL-dependent clearance rate of VLDL-TG, rather than reduced hepatic VLDL-TG synthesis or secretion [15]. ANGPTL3 also inhibits endothelial lipase, causing increased HDL cholesterol and phospholipids [16]. ANGPTL3 binds to adipocytes directly to activate lipolysis, releasing free fatty acids and glycerol [17]. Importantly, ANGPTL3 is proteolytically cleaved by proprotein convertases via recognition sequence at the position 221–224 (RAPR) to yield the N-terminal coiled-coil domain [18], which is sufficient and necessary for regulating plasma TG levels [18], whereas the C-terminal region containing the fibrinogen-like domain is important for angiogenesis [19].

4. ANGPTL4

ANGPTL4 was independently identified by several groups as a new ANGPTL family member that is a target of peroxisome proliferator-activated receptor and induced by fasting in adipocytes [20–22]. ANGPTL4 increases plasma TG levels at least in part through inhibiting LPL activity. Indeed, ANGPTL4-null mice have lower plasma TG and increased post-heparin plasma LPL activity, while overexpression of ANGPTL4, either whole body transgenic or liver-specific, increases plasma TG and decreases post-heparin plasma LPL activity [14]. As a typical ANGPTL family member, ANGPTL4 harbors an N-terminal coiled-coil domain and C-terminal fibrinogen like domain and a signal peptide that directs for secretion. Similar to ANGPTL3, ANGPTL4 is cleaved at the conserved proprotein convertase recognition sequence at position 161–164, RRKP, to release the N-terminal coiled-coil domain [23,24] that irreversibly inhibits LPL activity by disrupting LPL dimerization, converting the enzyme into inactive monomers [25]. Indeed, LPL produced from different types of cells, such as adipocytes, cardiomyocytes and macrophages, can be inhibited by ANGPTL4 [26]. Consistently, injection of a monoclonal antibody against the ANGPTL4 N-terminal domain in mice mimics phenotypes of ANGPTL4-null mice, such as low plasma TG levels [27,28].

Both ANGPTL3 and ANGPTL4 have been robustly linked to human plasma lipid profiles by various genome wide association studies [29]. Convincing evidence has been obtained by using new sequencing technology to show how sequence variations or loss-of-function mutations in the two genes affect lipid profiles. For instance, in about 2% of Caucasians, the glutamine residue at position 40 in ANGPTL4 is substituted by lysine, and this E40K substitution is associated with lower plasma TG and HDL-C concentrations [30,31]. By performing exome sequencing, Musunuru et al. found nonsense mutations in ANGPTL3 in two families with combined hypolipidemia, characterized by extremely low plasma levels of LDL-C, HDL-C and TGs [32]. By re-sequencing the coding regions, Romeo et al. found that 1% of the Dallas Heart Study population and 4% of those participants with a plasma TG in the lowest quartile have loss-of-function mutations in ANGPTL3, ANGPTL4 or ANGPTL5 [33].

5. Discovery of Lipasin in lipid regulation

Collaboration between Genentech and Lexicon created a library of mouse knockout models targeting genes encoding secreted and trans-membrane proteins, resulting in about 500 mouse mutant lines that were subjected to phenotypic screening. Gm6484 was one of the targeted genes, and Gm6484-null mice clearly have a phenotype of low serum TG levels [34]. Recently, 3 groups almost

Table 2
Expressions of Lipasin, ANGPTL3 and ANGPTL4 in response to various stimuli.

	Liver		WAT		BAT			Serum		References
	Fasting	Obesity[a]	Fasting	Obesity	Fasting	Obesity	Cold	Fasting	Obesity	
Lipasin	↓	↑	↓	↑	↓	↑	↑	↓[b]	NR	[8–11]
ANGPTL4	↑	––	↑	––	↑	––	↓	↑	↓	[21,22,38,39]
ANGPTL3	––	↑	NA	NA	NA	NA	NA	––	––	[40]

Lipasin and ANGPTL4 show opposite changes by fasting or cold exposure. WAT, white adipose tissue; BAT, brown adipose tissue; ↑, increase; ↓, decrease; ––, unchanged; NA, not applicable; NR, not reported.
[a] Diet-induced obesity or *ob/ob* mice.
[b] Serum Lipasin is induced by re-feeding following a fasting [11], and therefore it is likely reduced by fasting, although such time course has not been established.

simultaneously published papers on further characterization of this gene [8–11].

Ren et al. described the Gm6484 gene as RIFL (Re-feeding Induced in Fat and Liver) [10]. They showed that *RIFL* is highly induced during adipogenesis in 3T3 L1 cells and in primary cultures of murine and human adipocytes. Knockdown of *RIFL* resulted in reduced adipogenesis, characterized by reduced TG content. Mouse *RIFL* mRNA is highly enriched in white and brown adipose tissue (WAT and BAT) and liver, and its WAT levels are increased in *ob/ob* mice, a genetic obesity model that lacks leptin. In 3T3 L1 adipocytes, *RIFL* mRNA is induced by insulin and suppressed by agents that stimulate lipolysis, such as Dibutyryl-cAMP, forskolin and isoproterenol [10]. These data nicely showed several aspects of *RIFL* regulation and function in adipocytes.

Quagliarini et al. identified the Gm6484 gene product as ANGPTL8 in a homolog search for additional ANGPTLs, and showed that *ANGPTL8*, a paralog of ANGPTL3, is abundantly expressed in mouse liver, WAT, BAT and the adrenal gland. In humans *ANGPTL8* is expressed in liver, WAT and brain. A non-synonymous variant (R59W) was associated with lower plasma LDL-C and HDL-C in humans. Secreted from hepatocytes, circulating ANGPTL8 was detected in humans. Importantly, Quagliarini et al. showed that ANGPTL8 interacts with, and promotes the cleavage of ANGPTL3. Consistently, adenovirus-mediated overexpression of ANGPTL8 increases serum TG, but not in ANGPTL3-null mice. *ANGPTL8* expression is induced by food intake in an SREBP1c independently manner [11].

Our group identified Gm6484 as a nutritionally regulated gene by performing RNA-seq on mRNA of liver and fat from mice subjected to nutritional stimulations, including 24-h fasting and 3-month high-fat diet (HFD) treatment. We named the gene Lipasin, because of its lipoprotein lipase inhibition ('lipas' and 'in') activity and because 'in' denotes a circulating factor [8]. We showed that in mice *Lipasin* is highly enriched in liver and adipose tissues, especially BAT. In man, among 48 tissues that did not include BAT, *Lipasin* is predominantly expressed in liver. HFD treatment markedly increased *Lipasin* in liver, while 24-h fasting dramatically reduced its levels in fat. We later found that 48-h fasting also significantly reduced liver *Lipasin*. Injection of adenovirus-Lipasin in mice, causing overexpression of Lipasin in liver, increased serum TG levels by ~5-fold. We then generated recombinant Lipasin in *Escherichia coli*, and showed that at high concentrations, it inhibits LPL activity *in vitro* [8].

Because *Lipasin* is highly expressed in BAT, we examined whether *Lipasin* and other *ANGPTLs* that are abundant in BAT are thermoregulated. Cold exposure dramatically induced BAT *Lipasin*, while suppressed *ANGPTL4* expression. In addition, in response to fasting, Lipasin and ANGPTL4 always show opposite changes in liver, WAT, BAT and serum (Table 2) [9].

Different domains of ANGPTLs show distinct functions. In ANGPTL3, for example, the N-terminal coiled-coil domain and the C-terminal fibrinogen-like domain are involved in lipid regulation [18] and angiogenesis [19], respectively. Because Lipasin lacks the fibrinogen-like domain, we performed phylogenetic analysis using Lipasin and N-terminal domains of the 7 ANGPTLs. The 8 proteins were classified into 2 branches. Lipasin and ANGPTL3 were most closely related, and shared a common ancestor with ANGPTL4, which was then joined by ANGPTL5. The other branch contained ANGPTL 1, 2, 6 and 7. All the 4 proteins in the first branch, Lipasin, ANGPTL3, 4 and 5, are involved in serum TG regulation (Fig. 1). Lipasin, therefore, is a new but atypical ANGPTL family member, because of lacking the Fibrinogen-like domain, glycosylation sites and amino acids for forming disulfide bonds [9] (Table 1).

6. Perspective

The work from the 3 groups [8–11] constitutes a set that characterizes Lipasin functions from different aspects. The common theme is that Lipasin is a novel but atypical member of the ANGPTL protein family. Being highly enriched in the liver and nutritionally regulated, Lipasin is a hepatocyte-derived circulating factor that regulates plasma TG levels. Lipasin mRNA levels in liver and fat as well as its protein level in serum are reduced by fasting. Mice deficient for Lipasin have lower serum TG levels; conversely, its adenovirus-mediated overexpression increases serum TG levels, in part, through increased ANGPTL3 cleavage. *Lipasin* is induced during and involved in adipogenesis. In adipocytes, *Lipasin* is consistently up-regulated by insulin and down-regulated by agents that stimulate lipolysis, e.g., forskolin. *Lipasin* is thermoregulated in BAT; as cold exposure increases *Lipasin* but decreases ANGPTL4. In response to fasting, Lipasin and ANGPTL4 shows opposite changes, in terms of both mRNA levels in liver, WAT and BAT and protein levels in the serum.

There are discrepancies among these studies as well. The first is the expression pattern among tissues. In mice, we showed that Lipasin expression level is highest in liver and BAT, but much lower in WAT. Ren et al. and Quagliarini et al. showed comparable *Lipasin* expression levels in liver, BAT and WAT, and also in the adrenal gland. In humans, we showed that *Lipasin* is predominantly in the liver with very low expression in WAT, while Quagliarini et al. showed a relatively high expression in WAT and also in the brain. Because Lipasin levels are sensitive to nutritional status, these differences likely need to be resolved by using more tightly controlled feeding regimens.

Quagliarini et al. showed that Lipasin promotes cleavage of ANGPTL3, releasing the N-terminal domain involved in lipid metabolism. Because ANGPTL3 was previously shown to directly bind to adipocytes and activate lipolysis [17], if ANGPTL3's N-terminal domain is involved in this process, Lipasin is expected to increase adipocyte lipolysis. But Ren et al. showed that Lipasin is lipogenic. In other words, Lipasin by itself is lipogenic, but probably also promotes cleavage of ANGPTL3 to increase lipolysis. These two seemingly opposite effects on adipocytes need to be resolved

by future experiments, e.g., distinct functions of ANGTPL3 domains in adipocyte lipid metabolism.

The discovery of Lipasin, naturally, raises more questions than it answers. We therefore list here some potential questions to address:

(1) Can loss-of-function mutations of Lipasin cause human hypotriglyceridemia? Loss-of-function mutations in ANGPTL3, 4 or 5 were found in about 1% of the Dallas Heart Study population [33]. It is likely that a small percentage of population, especially those with hypotriglyceridemia can be explained by Lipasin non-sense mutations. Individuals or family members with Lipasin non-sense mutations will serve as a definite proof for Lipasin functions in humans.
(2) Are plasma Lipasin levels altered in obesity, diabetes or other pathological conditions? We showed that liver *Lipasin* is increased in mice with diet induced obesity, whether obesity increases serum Lipasin levels, in either mice or humans, however, has not been reported. Because higher plasma TG is correlated with higher body mass index [35], and because Lipasin increases plasma TG, an elevated plasma Lipasin level can be a potential mechanism for increased TG in human obesity.
(3) Can Lipasin antibodies have therapeutic effect? Monoclonal antibodies against ANGPTL4 lower plasma TG levels. It is likely that Lipasin neutralizing antibodies can have similar effect, because Lipasin-null mice have lower TG levels.
(4) Can Lipasin function in an endocrine and/or autocrine manner? Does Lipasin have receptors, and where are they expressed? In addition to hepatocytes, is Lipasin also secreted from adipocytes? If so, what are the percentages of circulating Lipasin that is derived from liver and adipocytes, or other tissues?
(5) Does Lipasin play a role in angiogenesis? Although Lipasin does not have the fibrinogen like domain that is involved in angiogenesis, because Lipasin promotes the cleavage of ANGPTL3, which is involved in this process, then is it possible that Lipasin also plays a role in angiogenesis?
(6) What are the transcription factors that control Lipasin transcription? Why do Lipasin and ANGPTL4 always show opposite changes in response to fasting? How are Lipasin, ANGPTL3 and ANGPTL4 coordinated in lipid regulation?

The list is apparently far from complete, and Lipasin should go beyond only promoting ANGPTL3 cleavage, by having ANGPTL3 independent functions. The name Lipasin can be interpreted as lipoprotein lipase inhibition (either directly or indirectly by facilitating ANGPTL3 cleavage) and as a circulating factor as denoted by 'in'. The cholesterol biosynthesis pathway has been clearly elucidated, leading to the development of Statins, inhibitors of HMG-CoA, which effectively reduce LDL-cholesterol and risk of cardiovascular diseases [36]. In contrast, effective pharmaceutical approaches for lowering plasma TG levels are lacking. Levels and activity of LPL have been demonstrated to be a rate limiting process for uptake of fatty acids that are derived from TGs [37]. Therefore, Lipasin, which inhibits LPL, is emerging as a critical lipid regulator and a potential drug target.

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