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Review

Genetic and Epigenetic Alterations of Lysophosphatidic Acid Receptor Genes in Rodent Tumors by Experimental Models

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Abstract: Lysophosphatidic acid (LPA) is a bioactive mediator and induces several biological effects, including cell proliferation, migration, morphogenesis and differentiation. LPA interacts with at least six G protein-coupled receptors (GPCRs), including LPA receptor-1 (LPA_1), LPA_2, LPA_3, LPA_4, LPA_5 and LPA_6. These receptors show different biological functions through the binding of LPA, depending on the type of cells. In human malignancies, a high level of LPA production was found in plasma and ascites in ovarian cancer cases. Moreover, aberrant expression levels of LPA receptor genes were detected in some cancer cells. Therefore, it is suggested that LPA receptors may be involved in the pathogenesis of tumor cells as well as LPA per se. Recently, we have reported that alterations of LPA receptor genes also occur in rodent tumors. In this review, we summarize the recent evidence in the investigations of LPA receptor alterations in rodent tumors by experimental models. (DOI: 10.1293/tox.24.143; J Toxicol Pathol 2011; **24**: 143–148)

Key words: LPA, LPA receptor, mutation, DNA methylation, rodent

Introduction

Lysophosphatidic acid (LPA) is a simple bioactive phospholipid consisting of a phosphate, a fatty acid and a glycerol[1,2]. It is present in all mammalian cells and tissues and is detected in serum, plasma, saliva and activated platelets[1–3]. LPA induces several cellular responses, such as cell proliferation, differentiation, morphogenesis, cell migration, platelet aggregation, secretion of cytokine and chemokine and protection from apoptosis[1–3]. Moreover, it is suggested that LPA may be also involved in the development of human diseases, including cancers[2,4]. In fact, a high level of LPA production was found in plasma and ascites from patients with ovarian cancers, and the plasma levels of LPA were also increased in chronic hepatitis C in association with liver fibrosis[1,5]. LPA enhanced malignant abilities of tumor cells, including cell growth, migration, invasion, production of angiogenic factors and tumorigenicity[2,4].

LPA interacts with G protein-coupled receptors (GPCRs) to induce various responses. So far, at least six LPA receptors have been identified[1–4]. Previously, aberrant expressions of LPA receptor genes have been also detected in human malignancies, such as ovary, colon and thyroid tumors[6–9]. Moreover, the exogenous *LPAR2* or *LPAR3*-expressing ovarian cancer cells increased malignant properties, such as cell migration, invasion and tumorigenicity[10]. Therefore, it has been demonstrated that LPA receptors may be involved in the acquisition of malignant potency of tumor cells as well as LPA per se. In our energetic studies, we have indicated that mutations and aberrant expressions due to DNA methylation of LPA receptor genes occur in rodent tumors induced by experimental models. Here, we review the current knowledge about LPA receptor gene alterations in rodent tumors and suggest an involvement of the LPA signaling pathway during carcinogenesis of rodents.

LPA Receptors

LPA acts as a biological mediator binding with LPA specific G protein-coupled transmembrane receptors, LPA receptors. So far, at least six LPA receptors have been identified, including LPA_1/EDG2, LPA_2/EDG4, LPA_3/EDG7, LPA_4/P2Y9/GPR23, LPA_5/GPR92 and LPA_6/P2Y5[1–4]. Additionally, GPR87 has been proposed as a candidate for a new LPA receptor[11]. LPA receptors couple with individual sets of G proteins (G_i, G_q and G12/13) and mediate various biologi-

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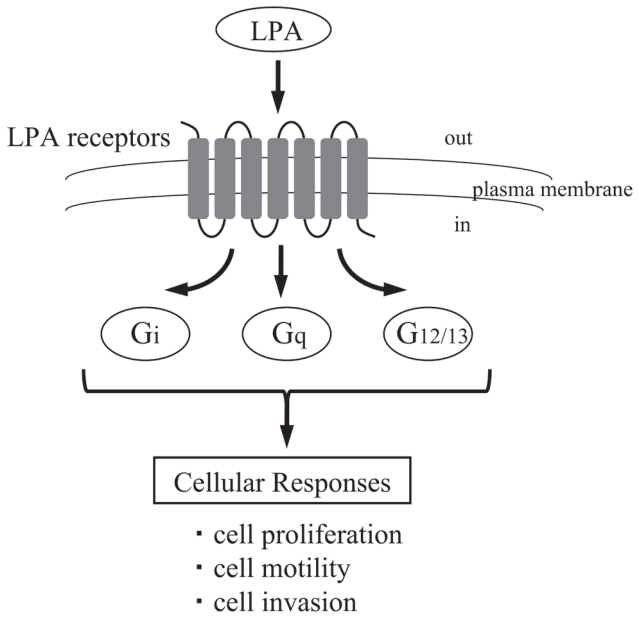

Fig. 1. Cellular responses of LPA receptors through the binding of LPA.

cal responses to LPA[1–4]. The expression patterns of LPA receptor genes are dependent on the type of cells[1]. While LPA_1 is ubiquitously expressed in normal tissues, the expressions of other LPA receptors are relatively restricted, suggesting that these receptors have different biological functions[1]. For example, both LPA_1 and LPA_2 can couple with Gi, Gq and $G_{12/13}$, and stimulates cell proliferation, phospholipase C activation, intracellular calcium mobilization and adenylyl cyclase inhibition[12]. However, some different phenotypes of LPA_1 and LPA_2 have also been found in knockout mice, indicating that LPA_1 and LPA_2, at least in part, possess distinct functions[13]. On the other hand, LPA_3 couples with Gi and Gq, but not $G_{12/13}$. In recent studies, LPA_6/P2Y5 has been identified as a causative gene of hair loss[14,15] (Fig.1).

Genetic Alterations

Mutations for LPA receptor genes were found in human colon cancer and osteosarcoma cells[16,17]. In colon cancer cells, 2 out of 6 cells showed *LPAR2* and/or *LPAR4* gene mutations[16]. *LPAR1* and *LPAR3* gene mutations occurred in one out of two osteosarcoma cells[17]. In rodent tumors, different patterns and frequencies of LPA receptor gene mutations were also observed.

Mutations of LPA receptor genes in rodent tumors

Lung: A mutation analysis for LPA receptor genes was performed using rat lung lesions induced by N-nitrosobis(2-hydroxypropyl)amine (BHP)[18,19]. This rat lung carcinogenesis model can obtain high yields of adenomatous lesions from preneoplastic lesions to carcinomas by continuous oral administration of BHP. Although no mutation of the *Lpar1* gene was found in 15 hyperplasias, two out of 12 adenomas (16.7%) showed a TGC to CGC (Cys to Arg) transition at codon 24 and a TAT to TAC (Tyr to Tyr) transition at codon 292, respectively. Moreover, the *Lpar1* gene mutations were detected in 7 out of 17 adenocarcinomas (41.2%). In the 7 adenocarcinomas, 3 cases showed an ACT to GCT (Thr to Ala) transition at codon 58, a CGG to CAG (Arg to Gln) transition at codon 241 and a CCC to TCC (Pro to Ser) transition at codon 308. All four other cases showed TTC to TCC (Phe to Ser) transitions at codon 295. These results suggest that mutations of the *Lpar1* gene may be involved in the acquisition of growth advantage from adenomas to adenocarcinomas during rat lung carcinogenesis induced by BHP[20]. By contrast, no mutation of the *Lpar2*, *Lpar3*, *Lpar4* and *Lpar5* genes was detected in this model[21].

Liver: To evaluate the *Lpar1* gene mutation during rat liver carcinogenesis, hepatocellular carcinomas (HCCs) were induced by exogenous and endogenous liver carcinogenesis models[22–24]. For the exogenous model, HCCs were induced by N-nitrosodiethylamine (DEN), which is one of the most well-known liver carcinogens. On the other hand, endogenous carcinogenesis means that tumors are induced by endogenous changes that occur without any established carcinogen exposure, and prolonged feedings of a choline-deficient L-amino acid-defined (CDAA) diet can induce rat HCCs[24]. Missense mutations of the *Lpar1* gene were detected in 7 out of 15 HCCs (46.7%) induced by DEN . By contrast, no mutation of *Lpar5* was found in HCCs induced by DEN. Five out of 12 HCCs (41.7%) induced by the CDAA diet showed missense mutations[25]. Therefore, it is suggested that the *Lpar1* mutations may play an important role in the development of rat HCCs induced by both liver carcinogenesis models.

Pancreas: A number of human pancreatic cancers commonly arise from pancreatic duct cells. Hamster pancreatic duct adenocarcinomas (PDAs) induced by the rapid production model using N-nitrosobis(2-oxopropyl)amine (BOP) histologically and genetically resemble the human situation[26,27]. In hamster PDAs induced by this model, only one out of 10 cases (10%) showed a GGA to GTA (Gly to Val) transversion at codon 355 of the *Lpar1* gene[28]. Therefore, it seems that the *Lpar1* gene mutation may be involved in a limited fraction of BOP-induced pancreatic duct carcinogenesis in hamsters.

Cancer cell lines: No mutation of the *Lpar1* gene was found in cell lines established from hamster PDAs induced by BOP[28]. In RLCNR rat lung adenocarcinoma cells, COS rat osteosarcoma cells, RH7777 rat hepatoma cells, B103 rat neuroblastoma and C6 glioma cells, no mutation of the *Lpar5* was detected[29] (Table 1).

Location of the Lpar1 gene mutations in rodent tumors

The *Lpar1* gene mutations were observed in several positions in rodent tumors. Particularly, the frequency of missense mutations of codon 295 was high. In lung adenocarcinomas induced by BHP, 4 out of 7 mutations were lo-

Table 1. Mutations of LPA Receptor Genes in Rodent Tumors

Organs/cells	Genes	Species	Carcinogens/ diets	Lesions/cell types	Incidences	Ref.
Lung	*Lpar1*	rat	BHP	adenomas	2/12 (16.7%)	(20)
	Lpar1	rat	BHP	adenocarcinomas	7/17 (41.2%)	(20)
	Lpar2	rat	BHP	adenocarcinomas	0/15 (0%)	(21)
	Lpar3	rat	BHP	adenocarcinomas	0/15 (0%)	(21)
	Lpar4	rat	BHP	adenocarcinomas	0/15 (0%)	(21)
	Lpar5	rat	BHP	adenocarcinomas	0/15 (0%)	(21)
Liver	*Lpar1*	rat	DEN	HCCs	7/15 (46.7%)	(25)
	Lpar1	rat	CDAA	HCCs	5/12 (41.7%)	(25)
	Lpar5	rat	DEN	HCCs	0/6 (0%)	(27)
Pancreas	*Lpar1*	hamster	BOP	PDAs	1/10 (10.0%)	(28)
Cell line	*Lpar1*	hamster		HPD (PDAs)	0/3 (0%)	(28)

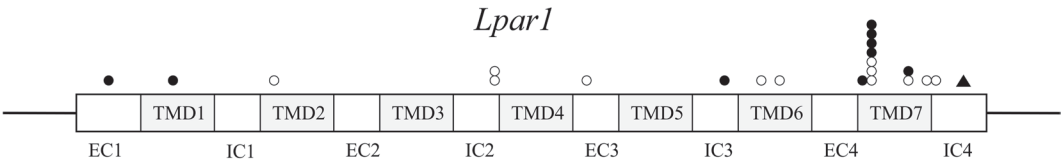

Fig. 2. The locations of *Lpar1* gene mutations in lung, liver and pancreatic tumors of rodents. EC, extracellular domain. TMD, transmembrane domain. IC, intracellular domain. ●, rat lung adenomas and adenocarcinomas;○, rat hepatocellular carcinomas; ▲, hamster pancreatic duct adenocarcinomas.

cated at codon 295[20]. In HCCs induced by exogenous and endogenous carcinogenesis, 3 out of 12 mutations were also found in codon 295[25]. The biological roles of mutations in codon 295 are unclear. However, codon 295 is adjacent to Lys294, which is one of the critical residues for ligand recognition or binding in the putative 7th transmembrane domain of LPA_1[30]. Recently, it has been reported that artificial replacement of Lys294 with Ala resulted in an enhancement of LPA response[31]. LPA_2 and LPA_3 contained basic amino acids equivalent to Lys294 of LPA_1. These amino acids are thought to play an important role in LPA binding and the subsequent receptor activation. Therefore, these findings suggest that the mutations in codon 295 of the *Lpar1* gene might affect the physical properties of the ligand recognition region in the 7th transmembrane domain, leading to changes in affinity of LPA binding or activation of signaling pathways upon LPA stimulation. The locations of *Lpar1* gene mutations in lung, liver and pancreatic tumors of rodents are shown in Fig. 2.

Patterns of the Lpar1 gene mutations in rodent tumors

In tumors induced by nitrosocompounds, the *Lpar1* gene mutation patterns have varied. Among 9 mutations in rat lung carcinogenesis model using BHP, 7 cases were T/A to C/G transitions and two were G/C to A/T transitions[20]. In DEN-induced rat HCCs, three T/A to C/G transitions, a C/G to T/A transition and three G/C to T/A transversions were observed[25]. In hamster PDAs induced by BOP, one mutation was a G/C to T/A transversion[28]. Although G/C to A/T transition is a common mutation pattern induced by nitrosocompounds[32,33], only two lung adenocarcinomas and one HCC showed this pattern. By contrast, all 5 mutations in rat HCCs by the CDAA diet were T/A to C/G transitions[25]. Oxy radical-mediated DNA damage generates several DNA adducts, including 8-hydroxyguanine and 8-hydroxyadenine[34]. It is well established that 8-hydroxyguanine induces G/C to T/A or A/T to C/G transversions in *Escherichia coli*[35]. Moreover, 8-hydroxyadenine also induces a T/A to C/G transition or an A/T to C/G transversion in mammalian cells[36,37]. Since T/A to C/G transition is a frequent pattern, it is suggested that the *Lpar1* gene may be a target for oxidative DNA damage in rodent tumors. In fact, it has been reported that oxidative stress is involved in rat hepatocarcinogenesis induced by the CDAA diet[38].

Epigenetic Alterations

As one of epigenetic mechanisms for the regulation of gene expression, DNA methylation of cytosine residues at CpG dinucleotides is a strong mediator for the gene silencing in mammalian genomes[39,40]. It has been reported that loss of tumor suppressor gene expressions is due to aberrant DNA methylation of gene promoter regions in several tumors[39,40]. In LPA receptor genes, distinct expression and DNA methylation patterns of LPA receptor genes were found in human colon cancer cells[16]. Aberrant DNA methylation statuses of LPA receptor genes were also detected in rodent tumors (Table 2).

Table 2. Aberrant DNA Methylation of LPA Receptor Genes in Rodent Tumors and Cancer Cell Lines

Organs/cells	Genes	Species	Carcinogens/diets	Lesions/cell types	DNA methylation	Ref.
Liver	*Lpar1*	rat	CDAA	livers (7d-20w)	unmethylated	(41)
	Lpar2	rat	CDAA	livers (7d-20w)	unmethylated	(41)
	Lpar3	rat	CDAA	livers (7d-20w)	methylated	(41)
		rat	CDAA	HCCs	methylated	(41)
	Lpar5	rat	DEN	HCCs	unmethylated	(29)
Lung	*Lpar5*	rat	BHP	adenocarcinomas	unmethylated	(29)
Cell line	*Lpar1*	rat		RH7777 (HCC)	methylated	(43)
		rat		B103 (neuroblastoma)	methylated	(43)
		mouse		B16F0 (melanoma)	methylated	(43)
		mouse		FM3A (breast cancer)	methylated	(43)
		mouse		L1210 (leukemia)	methylated	(43)
	Lpar3	mouse		LL./2 (lung cancer)	methylated	(43)
		mouse		B16F0 (melanoma)	methylated	(43)
		mouse		FM3A (breast cancer)	methylated	(43)
		mouse		L1210 (leukemia)	methylated	(43)
	Lpar5	rat		RH7777 (HCC)	unmethylated	(29)
		rat		RLCNR (lung cancer)	unmethylated	(29)

Liver

DNA methylation status and expression levels of the *Lpar1*, *Lpar2* and *Lpar3* genes were examined in a rat liver carcinogenesis model induced by the CDAA diet. The *Lpar1* and *Lpar2* genes were unmethylated in the livers of rats fed the CDAA diet for 7 days and 2, 12 and 20 weeks, as well as in normal liver tissues. In regard to the *Lpar3* gene, the livers at 7 days and 2 and 12 weeks were weakly or moderately methylated, and those at 20 weeks were markedly methylated, while normal liver tissues were unmethylated. Moreover, 4 HCCs induced by the CDAA diet were completely methylated. In those samples, the expression levels of the *Lpar1*, *Lpa2* and *Lpar3* genes were correlated with the DNA methylation status[41].

In regard to the *Lpar5* gene, 4 out of 6 HCCs induced by DEN were unmethylated, compared with normal liver tissues, which were methylated. These DNA methylation patterns were correlated with the *Lpar5* gene expression levels[29].

Lung

In rat normal lung tissues, the *Lpar5* gene was not expressed, and its DNA methylation status was methylated. By contrast, 5 out of 6 adenocarcinomas (83.3%) induced by BHP indicated increased expressions of *Lpar5* with an unmethylated status[29].

Cancer cell lines

While normal liver and brain tissues expressed the *Lpar1* gene and its DNA methylation status was unmethylated, RH7777 and B103 cells showed hypermethylation of the *Lpar1* gene with elevated expression levels[42]. In regard to the *Lpar5* gene, RLCNR and RH7777 cells were unmethylated compared with normal lung and liver tissues[29]. In mouse tumor cells, aberrant DNA methylation of the *Lpar1* gene was detected in B16F0 melanoma cells, FM3A mammary carcinoma cells and L1210 leukemia cells. The *Lpar3* gene in LL/2 lung tumor cells, B16F0, FM3A and L1210 cells was also hypermethylated compared with their adjacent normal tissues[43].

Conclusion

We here reviewed the genetic and epigenetic alterations of LPA receptor genes occurring in rodent tumors induced by experimental carcinogenesis models and cancer cell lines, demonstrating that alterations of the LPA signaling pathway may be involved in the development of tumor cells in rodents. Our successive study demonstrated that an artificial mutated form of LPA_1 that lacked the carboxyl terminal was constitutively active and oncogenic[44]. Therefore, it is suggested that LPA receptors may be one of the important molecules for the development of anticancer and chemoprevention agents in clinical cancer approaches.

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