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HUMAN GENETICS • REVIEW

PLAGL1: an important player in diverse pathological processes

Ana F. Vega-Benedetti[1] **· Cinthia Saucedo**[1] **· Patrizia Zavattari**[2] **· Roberta Vanni**[2] **·
José L. Zugaza**[3,4,5] **· Luis Antonio Parada**[1]

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Abstract The *PLAGL1* gene encodes a homonymous zinc finger protein that promotes cell cycle arrest and apoptosis through multiple pathways. The protein has been implicated in metabolic, genetic, and neoplastic illnesses, but the molecular mechanisms by which the protein PLAGL1 participates in such diverse processes remains to be elucidated. In this review, we focus mainly on the molecular biology of *PLAGL1* and the relevance of its abnormalities to several pathological processes.

Keywords PLAGL1 · Tumor suppressor gene · Cancer · Diabetes

Introduction

The pleomorphic adenoma gene-like 1 (*PLAGL1*) gene encodes a zinc finger protein which plays roles as transcription factor as well as cofactor of other regulator proteins. The gene was originally identified by Abdollahi et al. (1997a), using growth stress to induce malignant transformation of rat ovarian cells cultured in vitro, they identified a transcript which was expressed in normal cells but not in the related transformed cells. The gene, at the time, was named *Lot1* for "lost on transformation-1". Likewise, the mouse ortholog (*Zac1*) was identified in a model of pituitary tumorigenesis (Spengler et al. 1997). Since the human gene has frequently been detected downregulated in various tumor types, including ovarian, breast, prostate, and colon cancer (Cvetkovic et al. 2004; Jacobs et al. 2013; Kowalczyk et al. 2015; Li et al. 2014; Ribarska et al. 2014), it is considered a tumor suppressor gene, whose product regulates apoptosis and cell cycle arrest. We discuss here the structural and functional aspects of this gene, as well as the involvement of its abnormalities in developmental diseases and cancer.

Gene and protein structure

The human gene, *PLAGL1*, was identified by nucleotide sequence alignment because of its similarity with *PLAG1*, the prototype of a family of genes encoding zinc finger proteins (Kas et al. 1998). Also, screening the human fetal kidney cDNA library of the Merck/Washington University EST project, the researchers isolated two cDNA clones which corresponded to two splice variants of *PLAGL1*. Furthermore, genomic analysis demonstrated that these clones derived from the same gene, which had high similarity with the originally identified rat ortholog *Lot1* (GenBank U72621), and the two splicing isoforms represented differences in the 5′ non-coding region (Kas et al. 1998). The gene (GenBank U81992) was mapped, by FISH analysis, to chromosome band 6q24 (Varrault et al. 2001). The mouse ortholog of

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✉ Luis Antonio Parada
lparada@unsa.edu.ar

[1] Institute of Experimental Pathology, UNSa-CONICET, Ave. Bolivia 5150, 4400 Salta, Argentina

[2] Biochemistry, Biology and Genetics Unit, Department of Biomedical Sciences, University of Cagliari, Cittadella Universitaria di Monserrato SP 8, Km 0.700, 09042 Monserrato, Cagliari, Italy

[3] IKERBASQUE, Basque Foundation for Science, 48011 Bilbao, Spain

[4] Achucarro Basque Center for Neuroscience, Bizkaia Science and Technology Park, Building 205, Zamudio, Spain

[5] Department of Genetics, Physic Anthropology and Animal Physiology, Faculty of Medicine and Dentistry, University of the Basque Country, Barrio Sarriena s/n, 48940 Leioa, Spain

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PLAGL1, *Zac1*, was isolated from a plasmid library constructed from whole pituitary tissue from BALB/c mice. The clone, designated B-16, contained a 3.7-kb insert encoding a 667 amino acids protein with a predicted molecular weight of 75 kDa (Spengler et al. 1997). Subsequently, Huang and Stallcup (2000) isolated a variant of *Zac1*, which contains a 33-nucleotide insert located after codon 567 of the previously reported *Zac1* sequence, and encoding for the amino acids PQMQLQPLQLQ (AF147785) (Huang and Stallcup 2000). The mouse gene was mapped to the proximal region of chromosome 10 (10A2), a region that shares high homology with human chromosome band 6q24 where *PLAGL1* localizes (Piras et al. 2000). The rat ortholog, initially named *Lot1*, was mapped to chromosome 1 (Abdollahi et al. 1997a).

The gene exhibits a high degree of conservation among species, both at the nucleotide and amino acid sequences levels. The comparison between *Lot1* and *PLAGL1* revealed 76.4 % similarity in their nucleotide sequence, whereas the *Zac1* coding sequence is 74.6 % identical to the human gene. The three variants encode a seven zinc fingers (ZF) protein, composed of 463 (PLAGL1), 693 (ZAC1), and 583 (LOT1) amino acid residues, with calculated molecular weights of 54 kDa, 75 kDa, and 66 kDa, respectively. There is 67.7 % identity at the amino acid level between PLAGL1 and ZAC1, and the ZF regions show 85.5 % identity (Abdollahi et al. 1997b). The rat protein has 68.5 % similarity at the amino acid level with the human gene product, while their ZF domains are similar in their 84.2 % amino acid sequence (Varrault et al. 1998). ZAC1 differs from LOT1 and PLAGL1 in the central region characterized by 34 proline repeats, typically PLE, PMQ, and PML, and ZAC1 and LOT1 differ from PLAGL1 by the C-terminal region, rich in proline, glutamine, and glutamic acid amino acids. The linker domain, the region flanking the DNA-binding domain, exists in mice, humans, and rats, and confers transactivation in conjunction with the proline repeat domain (PR) (Fig. 1). The N-terminal part of the C terminus contains the coactivator binding domain (CB) that can recruit the general coactivators p300/CBP (Theodoropoulou et al. 2010). DNA binding occurs through ZF 2, 3, 4, 6, and 7, while the activity of PLAGL1 on transcription is controlled by the interactions of ZF 6 and 7 with the coactivators p300 and PCAF (Hoffmann et al. 2003; Theodoropoulou et al. 2010) (Fig. 1).

PLAGL1 gene transcription regulation

PLAGL1 is an imprinted gene, which is exclusively expressed from the paternal allele in various tissues during development (Kamiya et al. 2000). Like the vast majority of imprinted genes, a differentially methylated region (DMR), rich in CpG sequences, is involved in the regulation of its transcription. An in vitro model for *PLAGL1* gene regulation demonstrated that methylation of the CpG islands induces heterochromatin modification that represses gene transcription (Varrault et al. 2001). Apart from the P1 promoter, *PLAGL1* transcripts can also originate from two additional promoters; P2-*PLAGL1* mapping upstream of P1, in an unmethylated CpG island, and P3 located in a unique promoter region that yields transcripts including only the last three exons of the gene (Iglesias-Platas et al. 2012). In addition to these three alternative transcripts, the *PLAGL1* region encodes two non-coding RNAs (ncRNAs), *HYMAI* and *PLAGL1it*, important in recruiting histone methyltransferases to promoters and interacting with chromatin (Iglesias-Platas et al. 2012). Interestingly, Czubryt et al. (2010) have identified a putative binding site for the transcription factor MEF2 −2400 to −1400 bp upstream of the first coding exon of the mouse, rat, and human genes. Binding of MEF2 to this sequence has been demonstrated by ChIP assay and its function as a promoter with a luciferase reporter. However, no mRNAs originating from this putative promoter has been demonstrated by 5′RACE analysis (Czubryt et al. 2010).

PLAGL1 gene expression

PLAGL1 has been shown to be widely expressed in both adult and fetal human tissues, although with differences among them. The highest levels of expression in fetal tissues were observed in the kidney, lung, heart, and spleen, whereas peripheral leukocytes, liver, skeletal muscle, and brain exhibit low *PLAGL1* expression levels in adults (Varrault et al. 1998). Godlewski et al. (2015) investigated the expression profile of the PLAGL1 protein in normal kidney tissue collected from patients who underwent nephrectomy due to renal cell carcinoma. Their data clearly showed that such differences in protein expression not only occur between tissues, but also within an organ. In the kidney, a high expression level was detected in ascending limbs of Henle's loop, distal tubules, and collecting ducts, whereas the proximal tubules and renal corpuscles exhibited moderate and weak, respectively, PLAGL1 expression (Godlewski et al. 2015). Similarly, the expression of *PLAGL1* varies during keratinocyte differentiation. It is abundantly expressed in cells at early stages, and it gradually disappears as cells migrate towards the outermost layer of the skin (Basyuk et al. 2005).

In the mouse, *Zac1* regulates growth and differentiation during embryogenesis through multiple mechanisms. Valente et al. (2005) performed an extensive *Zac1* gene expression analysis simultaneously with the expression of PCNA, FORSE-1, β-tubulin, D-28k, and Netrin1, all markers of cell proliferation and differentiation, in mice at different developmental stages. They found that, in embryonic stages, there is a strong *Zac1* expression in neuroephitelia, cartilage primordium sites, and skeletal muscle precursor cells.

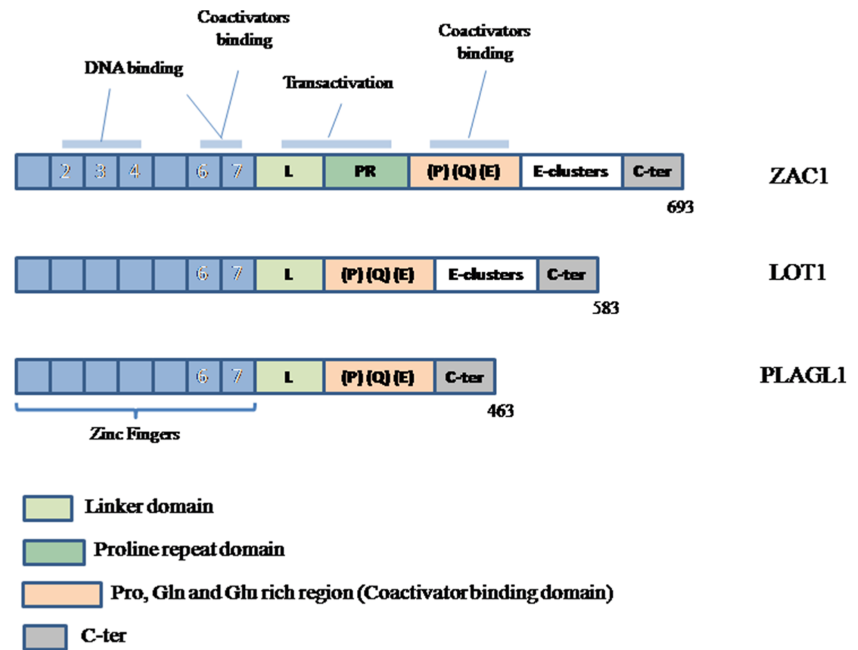

Fig. 1 Scheme of ZAC1/LOT1/PLAGL1 proteins. The numbers denote amino acids and domains are boxed. The three proteins contain identical zinc finger domains (ZF) that have a role in DNA binding. The linker (L) domain confers transactivation in conjunction with the proline repeat (PR) domain, which exists only in the mouse protein. The N-terminal part of the C terminus contains the coactivator binding domain

However, the expression level gradually decreases in ossification sites, differentiated skeletal muscle, and neuronal cells after birth and onwards, and only persists in some brain areas (Valente and Auladell 2001; Valente et al. 2005).

With respect to the expression of the rat ortholog, *Lot1*, high mRNA levels have been detected in the ovary, pancreas, testes, and uterus, whereas brain and kidney tissues exhibit low levels, and has not been detected transcription in heart, liver, lung, spleen, and skeletal muscle (Abdollahi et al. 1997a).

PLAGL1 function

PLAGL1 acts as a transcription factor and cofactor of other proteins and nuclear receptors (Fig. 2). It regulates the transcription of, among others, $p21^{WAF1/Cip1}$, a cyclin-dependent kinase (cdk) inhibitor, *PPAR*γ (peroxisome proliferator-activated receptor γ), and $PACAP_1$*-R* (type I pituitary-adenylate-cyclase-activating polypeptide receptor), through differential DNA binding (Barz et al. 2006; Ciani et al. 1999; Hoffmann et al. 2003). Hoffmann et al. (2003) have studied the interactions between all ZFs in PLAGL1 with DNA. PLAGL1 binds to G_4C_4 palindromic DNA element as a monomer primarily through ZFs 6 and 7, conferring transactivation function, but similar binding to repeat elements turns the protein into a repressor. Furthermore, dimerization through ZF 2 induced G_1 arrest, when binding to genes containing the repeat element, or apoptosis, when binding to palindromic elements (Hoffmann et al. 2003).

PLAGL1 modulates cell cycle progression through convergent pathways (Fig. 2). On one hand, it interacts with p53, binding to its N-terminal region or weakly to its C-terminal fragment. Huang et al. (2001) tested the effect of PLAGL1 in different cell lines lacking p53 or expressing functional p53, and their findings support the notion that coactivator function of PLAGL1 requires the presence of functional p53 protein. Both proteins induce the expression of the receptor for pituitary adenylyl cyclase-activating peptide ($PACAP_1$-R). Ligands binding to $PACAP_1$-R induce gene transcription through activator protein 1 (AP-1), essential for proliferation and differentiation of some cell types and maybe for programmed cell death (Huang et al. 2001). Moreover, PLAGL1 and p53 induce $p21^{WAF1/Cip1}$ that blocks cyclin A transcription, causing G_1 arrest. Considering this interaction, PLAGL1 is recruited by p73, a member of the p53 family, to the *p21* gene promoter. PLAGL1 stabilizes the association between the coactivators p300 and PCAF in the complex and regulates the histone acetyltransferase activity (HAT) of PCAF by inducing histone H4 acetylation and, in this way, through p73, the transcription of *p21* (Hoffmann et al. 2006; Hoffmann and Spengler 2008). On the other hand, PLAGL1 regulates *p21* promoter through a p53 independent pathway. It interacts with amino acids 481–683 of Sp1, a zinc finger protein that is also involved in the modulation of *p21* transcription through the formation of Sp1–Sp1 and Sp1–PLAGL1 complexes that interact with Sp1- or PLAGL1-responsive elements, inducing *p21* transcription. Conversely, histone deacetylase 1 (HDAC1) interacts and competes with PLAGL1 for this complex formation and represses *p21* transcription by blocking PLAGL1 activity (Liu et al. 2008, 2011). PLAGL1 also interacts with AP-1, at amino acids 1–344 in c-Jun and amino acids 1–101 in c-Fos (Wang et al. 2011). These functional interactions depend on the promoter

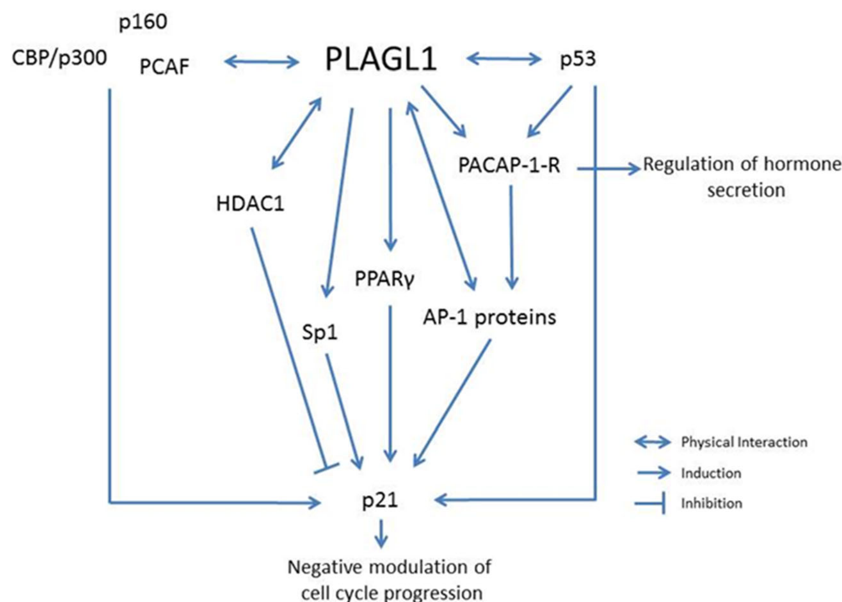

Fig. 2 Schematic diagram showing the interactions of PLAGL1 with partner proteins. PLAGL1 interacts with TP53 and the complex promotes the transcription of *p21*, producing the homonymous protein, which blocks the progression of the cell cycle. Additional partners include PCAF, HDAC1, and PPARγ, and interaction with them leads to a similar outcome

of AP-1 target genes, such as *p21*. PLAGL1 may regulate its transactivation activities, although AP-1 regulation is complex and depends on different events, such as post-translational modifications. Wang and coworkers observed that c-Jun enhances *p21* induction by PLAGL1 in HeLa/p53 shRNA cells after treatment with PMA, a tumor promoter (Liu et al. 2011; Wang et al. 2011). Moreover, PLAGL1 induces the expression of PPARγ, which inhibits cell cycle progression through $p21^{Cip1}$ induction (Liu et al. 2011; Theodoropoulou et al. 2010). Not only does PPARγ stimulation induce apoptosis through the caspase-dependent pathway and the extrinsic pathway, but its inhibition also leads to anchorage-dependent apoptosis (anoikis) (Wu et al. 2012). More recently, the *Tcf4* gene was identified as another direct target of PLAGL1. Experiments in a cell model of mouse neurogenesis demonstrated that overexpression of *PLAGL1* induces transactivation at the *Tcf4* gene locus, leading to p57-mediated cell cycle arrest in G1 (Schmidt-Edelkraut et al. 2014). Interestingly, PLAGL1 also regulates hormone secretion and metabolism in adipose tissue. The $PACAP_1$*-R* gene is induced by PLAGL1 and p53 and produces a protein that regulates pituitary hormone, catecholamine, and insulin secretion in the mature endocrine system (Ciani et al. 1999; Theodoropoulou et al. 2010). Other studies, performed with rats, have recently shown that PLAGL1 is highly expressed in white adipose tissue and that androgens inhibit its expression (Mirowska et al. 2014).

PLAGL1 gene in disease

As an imprinted gene, *PLAGL1* plays an important role in fetal growth control during development, metabolism, and cell proliferation. Genetic and epigenetic alterations of this gene have been linked to the physiopathology of transient neonatal diabetes mellitus (TNDM), Beckwith–Wiedemann syndrome (BWS), and cancer (Table 1).

TNDM is a metabolic disease characterized by intrauterine growth retardation, hyperglycemia, and low or undetectable level of insulin during the first 6 months of life (Kamiya et al. 2000). Overexpression of *PLAGL1* is a factor playing a fundamental role in the pathology of this disease, and an increase in the transcriptional level of the gene has been demonstrated to be associated with chromosome arm 6q structural and functional anomalies (Kamiya et al. 2000). This overexpression may be the consequence of paternal uniparental isodisomy, i.e., the inheritance of two identical copies of the paternal allele (active). The disease may also develop as a consequence of epigenetic alterations at the TNDM locus, leading to imprinting failure, which results in the activation of the maternal allele, which is normally not expressed during development (Kamiya et al. 2000). Baglivo et al. (2013) have elegantly elucidated the molecular mechanism by which this epigenetic change causes TNDM. They found that the zinc finger protein 57 (ZFP57) binds to the DNA sequence of the imprinting control region (ICR) of the *PLAGL1* gene in a methylation-dependent manner and that the lack of methylation on the maternal allele reduces the recruitment of the repressor ZFP57/KAP1 complex on this locus, allowing its transcription. Furthermore, mutations *PLAGL1*-R248H (ZF3) and -H277N (ZF4) affect the binding of ZFP57 to methylated DNA, and such changes are also associated with TNDM (Baglivo et al. 2013).

The increased level of PLAGL1 protein in fetal and neonatal stages causes reduction in the β-cell mass of the pancreas, by apoptosis or cell cycle arrest, which, in turn, impairs

Table 1 *PLAGL1* gene alterations in human pathology

Disease	Molecular change	Reference
TNDM[a]	Uniparental isodisomy (paternal) P1 abnormal methylation	Kamiya et al. (2000)
BWS[b]	P1 hypomethylation, associated to imprinting abnormalities at chromosome band 11p15.5	Bliek et al. (2009); Milani et al. (2014)
Ovarian cancer	Loss of heterozygosity (LOH) Low mRNA and protein levels	Abdollahi et al. (1997a)
NFPA[c]	Loss of heterozygosity (LOH) Low mRNA and protein levels	Pagotto et al. (2000)
Diffuse large B-cell lymphoma	Abnormal transcription from P2	Valleley et al. (2010)
Prostate cancer	Gene deletion/P1 hypermethylation	Jacobs et al. (2013; Ribarska et al. (2014)
Gastric cancer	Gene deletion/P1 hypermethylation	Li et al. (2014)
Colorectal cancer	Low expression	Kowalczyk et al. (2015)

[a] Transient neonatal diabetes mellitus

[b] Beckwith–Wiedemann syndrome

[c] Non-functioning pituitary adenoma

insulin sensitivity and release (Aguilar-Bryan and Bryan 2008). Studies have demonstrated that PLAGL1, at the molecular level, controls the transcription of *PPARγ*, *PACAP1-R*, and *Rasgrf1*, which encode proteins closely related to the carbohydrate metabolism (Barz et al. 2006; Hoffmann and Spengler 2012). PLAGL1 induces the expression of PPARγ and PACAP1-R in the pancreatic islets, inhibitors of β-cell proliferation and insulin secretagogue, respectively (Barz et al. 2006; Kamiya et al. 2000). On the contrary, the guanine nucleotide exchange factor (*Rasgrf*) reduced its expression and activated signaling pathways that led to insufficient insulin secretion (Hoffmann and Spengler 2012). The PLAGL1 protein also regulates the expression of the *Glut4* gene. This gene encodes a protein that functions as a glucose transporter, and it has been demonstrated in rat cardiomyocytes that overexpression of *PLAGL1* leads to increased level Glut4 and increased glucose uptake in this cell type (Czubryt et al. 2010). These results are somehow paradoxical; on one hand, overexpression of the PLAGL1 protein may stimulate glucose clearance from the bloodstream, but on the other hand, high levels of this protein provoke hyperglycemia in TNDM patients. Interestingly, *PLAGL1* has also been included among the approximately 50 susceptibility genes for type 1 diabetes (Hisanaga-Oishi et al. 2014). T-cells isolated from non-obese mice affected by this disease exhibited higher growth/survival ratios, but associated with low PLAGL1 expression level, compared to T-cells from non-diabetic mice (Hisanaga-Oishi et al. 2014). It is likely that this proliferative capacity is related to the autoimmune destruction of the pancreatic β-cells that characterize type 1 diabetes.

PLAGL1 is also involved in the pathology of BWS, a genetic disorder characterized by body overgrowth, visceromegaly, abdominal wall defects, and, in some cases, neonatal hypoglycemia, cardiac defects, and/or mental retardation. Moreover, patients with BWS are susceptible to develop cancer, especially hepatoblastoma and Wilms' tumor (Choufani et al. 2010). The syndrome develops, basically, as a consequence of genetic alterations or imprinting abnormalities at chromosome band 11p15.5, where the *IGF2*, *H19*, *CDKN1C*, and *KCNQ1OT1* genes map (Soejima and Higashimoto 2013). The most common cause is loss of methylation at the ICR2/KvDMR1 within this chromosome region. However, in 2009, it was reported that some BWS patients show also hypomethylation at the ICR of other maternally methylated genes, such as *MEST*, *GNAS*, *IGF2R*, and *PLAGL1*, suggesting that the participation of multiple imprinted loci, and their possible molecular interactions, are likely responsible for the variation in the typical clinical presentation of BWS (Bliek et al. 2009; Milani et al. 2014). Bliek et al. (2009) studied a large cohort of BWS patients and found that four patients displayed complete or near-complete hypomethylation of the *PLAGL1* ICR. Furthermore, Tee et al. (2013) have reported that a high percentage of BWS patients who had loss of methylation at the *PLAGL1* ICR were conceived after assisted reproductive technology. As previously mentioned, this epigenetic change is normally associated with TNDM. However, these patients neither suffered from neonatal diabetes nor were they macrosomic. Therefore, these authors suggested that *PLAGL1* hypomethylation may alter the clinical presentation of BWS (Tee et al. 2013).

Given the role in cell proliferation and survival, it is not surprising that altered expression of *PLAGL1* has been linked to cancer (Table 1). The role of *PLAGL1* in cancer was demonstrated almost two decades ago by Abdollahi et al. (1997a),

when *Lot1* gene transcription was detected in all non-tumorigenic rat ovarian cell lines; however, it was absent or decreased in neoplastic cells (Abdollahi et al. 1997a). *PLAGL1* is considered a tumor suppressor gene, which controls cell cycle progression by regulating p21 expression, and supporting evidence for its role as a tumor suppressor gene comes from early transfection assays with constructs encoding PLAGL1 on human osteosarcoma cell lines, which resulted in growth inhibition and cell death after 24 h of treatment (Varrault et al. 1998). Investigations that aimed to elucidate the molecular mechanisms by which this gene contributes to tumorigenesis showed that functional alterations are more frequent than structural changes. In fact, point mutations have been detected in only 71 out 21,029 (0.3 %) samples from almost all kinds of human tumors, according to the Catalogue of Somatic Mutations in Cancer (COSMIC, http://cancer.sanger.ac.uk/cosmic), whereas, and consistent with the study performed on ovarian cell lines, loss of heterozygosity (LOH) at the genomic region where *PLAGL1* maps (6q24) and low or undetectable mRNA levels were found in 40 % of ovarian and breast cancers samples (Cvetkovic et al. 2004).

Pagotto et al. (2000) demonstrated LOH at the *PLAGL1* locus, and reduced mRNA and protein levels also occur in non-functioning pituitary adenoma (NFPA). More recently, Vieria Neto et al. (2013) confirmed that the *PLAGL1* transcriptional level was significantly lower in NFPA compared to that of somatotropinomas (another pituitary adenoma subtype) and normal pituitary tissue. NFPA is a devastating tumor type because of its high rate of recurrence. Interestingly, NFPA expressing high levels of *PLAGL1* were inversely associated with recurrence (Noh et al. 2009). Moreover, studies performed with a pituitary tumor cell model demonstrated that treating the cells with octreotide, a somatostatin analog, increases PLAGL1 expression and produces cell cycle arrest (Theodoropoulou et al. 2006, 2009). Diffuse large B-cell lymphoma, an aggressive form of non-Hodgkin's lymphoma, is another tumor type in which low expression levels of PLAGL1 have been detected (Valleley et al. 2010). However, these authors discovered that the molecular event most frequently associated with such change was loss of transcription from promoter P2, rather than LOH at the genomic region 6q24 and/or abnormal P1 promoter methylation. On the contrary, both deletion and promoter hypermethylation of the *PLAGL1* gene have been frequently detected in samples from prostate and gastric cancers (Jacobs et al. 2013; Li et al. 2014; Ribarska et al. 2014). Downregulation of *PLAGL1* gene expression was also demonstrated in tumor tissues from patients with colorectal cancer, and the low mRNA and protein levels correlated with the evolution of metastatic disease (Kowalczyk et al. 2015).

Conclusion

In conclusion, *PLAGL1* has been found to be involved in metabolic, genetic, and neoplastic illnesses, but the molecular mechanisms by which it participates in this diverse set of diseases still remain to be elucidated.

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Compliance with ethical standards

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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