Cancer Immunology, Immunotherapy (2019) 68:1039–1058
https://doi.org/10.1007/s00262-019-02350-8

REVIEW

PLAC1: biology and potential application in cancer immunotherapy

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Received: 22 August 2018 / Accepted: 24 May 2019 / Published online: 5 June 2019

Abstract

The emergence of immunotherapy has revolutionized medical oncology with unprecedented advances in cancer treatment over the past two decades. However, a major obstacle in cancer immunotherapy is identifying appropriate tumor-specific antigens to make targeted therapy achievable with fewer normal cells being impaired. The similarity between placentation and tumor development and growth has inspired many investigators to discover antigens for effective immunotherapy of cancers. Placenta-specific 1 (PLAC1) is one of the recently discovered placental antigens with limited normal tissue expression and fundamental roles in placental function and development. There is a growing body of evidence showing that PLAC1 is frequently activated in a wide variety of cancer types and promotes cancer progression. Based on the restricted expression of PLAC1 in testis, placenta and a wide variety of cancers, we have designated this molecule with new terminology, cancer–testis–placenta (CTP) antigen, a feature that PLAC1 shares with many other cancer testis antigens. Recent reports from our lab provide compelling evidence on the preferential expression of PLAC1 in prostate cancer and its potential utility in prostate cancer immunotherapy. PLAC1 may be regarded as a potential CTP antigen for targeted cancer immunotherapy based on the available data on its promoting function in cancer development and also its expression in cancers of different histological origin. In this review, we will summarize current data on PLAC1 with emphasis on its association with cancer development and immunotherapy.

Keywords Cancer · Development · Immunotherapy · PLAC1 · Placenta

Abbreviations

ADC	Antibody-drug conjugate
C/EBPβ-2	CCAAT/enhancer-binding protein β-2
CRC	Colorectal cancer
CTA	Cancer–testis antigen
CTB	Cytotrophoblast
CTP	Cancer–testis–placenta
d.p.c	Days post coitum
EGF	Epidermal growth factor
EMT	Epithelial–mesenchymal transition

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ERα	Estrogen receptor alpha
EVT	Extravillous trophoblast
ICC	Immunocytochemistry
IF	Immunofluorescence
IHC	Immunohistochemistry
KGF	Keratinocyte growth factor
LT	Large T antigen
LXR	Liver X receptor
miR	MicroRNA
NICD	Notch1 intracellular domain
Non-X CTA	Non-X chromosome-linked cancer–testis antigen
NSCLC	Non-small cell lung cancer cells
PLAC1	Human placenta-specific 1 gene/transcript
PLAC1	Human placenta-specific 1 protein
Plac1	Mouse placenta-specific 1 gene/transcript
Plac1	Mouse placenta-specific 1 protein
PPARδ	Peroxisome proliferator-activated receptor-δ
PR	Progesterone receptor
PTM	Post-translational modification
RB	Retinoblastoma protein
RT-qPCR	Reverse transcription–quantitative polymerase chain reaction
RXRα	Retinoid X receptor alpha
siRNA	Small interfering RNA
SOX2	SRY-related HMG 2 box
Sp1	Specificity protein 1
STB	Syncytiotrophoblast
TAA	Tumor-associated antigen
Tp53	Human tumor protein p53
TSA	Tumor-specific antigen
WB	Western blot
X-CTA	X chromosome-linked cancer–testis antigen
ZP	Zona pellucida

Introduction

A major health complication, cancer is one of the leading causes of mortality in the world. Uncontrolled growth of malignant tumors creates damage and functionally impairs normal tissues. Defects in the regulation of cell proliferation, resistance to apoptotic death, ability to invade host tissues, metastasis to distant sites and evasion of host immune defense mechanisms reflect the malignant phenotype of cancers [1].

To date, various "target non-specific strategies" ranging from surgery and radiotherapy to chemotherapy have been employed to overcome cancer. All these therapeutic modalities are associated with off-target effects in normal tissues as well, albeit to different degrees. With the emergence of immunotherapy and taking advantages of efficacy and specificity of the immune system, the paradigm of cancer treatment has been revolutionized during the past two decades. However, a major obstacle in cancer immunotherapy is targeting tumor-specific antigens, making targeted therapy achievable with less normal cell impairment.

In 2009, the US National Cancer Institute proposed the criteria for prioritization of cancer antigens based on functional importance including therapeutic function, immunogenicity, role of the antigen in oncogenicity, specificity, expression level and percentage of antigen-positive cells, stem cell marker expression, number of patients with antigen-positive cancers, number of antigenic epitopes, and cellular location of antigen expression [2]. Up to now, there is no ideal cancer antigen possessing all criteria mentioned above despite tremendous efforts that are being made to discover new tumor targets achieving the most desirable properties for cancer immunotherapy. Tumor-associated antigens (TAAs) [3], tumor-specific antigens (TSAs) [3, 4], and cancer–testis antigens (CTAs) [5, 6] are the main classes of tumor antigen categories employed so far for targeted immunotherapy of cancers. TAAs are expressed in both normal and cancer cells. TSAs are expressed in tumor cells but not in normal cells and CTAs are expressed in gametes and trophoblasts and in many types of cancer but not in normal somatic cells [7]. Due to the fact that TAAs are expressed in tumor tissues as well as normal cells, their targeting might result in autoimmune diseases and immunologic tolerance [5, 8]. Although TSAs are bona fide targets for cancer immunotherapy and their discovery is quite critical, unfortunately, the number of true TSAs is rather limited. Based on the expression profile of CTAs, there is less concern in targeting them due to their limited expression in healthy tissues, their expression in non-critical tissues or being present in a limited period of life. In this regard, CTAs have been the focus of many researchers in the field of cancer biology/immunotherapy during the past couple of years with encouraging results [6, 9].

Many similarities exist between placentation and growth of cancer cells. In a manner similar to malignant cells, placental trophoblasts migrate within the uterus and invade the vasculature to nourish the developing fetus [10]. Downregulation of the immune system is another feature of placentation common with most cancers [11]. As with many cancer types, in contrast to the majority of human somatic tissues, human placenta displays telomerase activity [12]. Another similarity between normal placentation and cancer is the existence of aneuploidy during placenta and cancer development [13] even with such epigenetic changes as selective hypermethylation [14]. Therefore, identifying common antigens between the placenta and cancer cells could be viewed as one justifiable approach to discovering antigens for effective immunotherapy of cancers. This point of view was initially presented by Dr. John Beard in the late nineteenth

century who proposed the idea of similarity between tumors and placentation [15] which was later reiterated by Dr. Lloyd Old who presented his idea of "Cancer is a Somatic Cell Pregnancy" in an editorial in 2007 [16].

Placenta-specific 1 (*PLAC1*) is one of oncoplacental genes introduced by Cocchia et al. [17]. It is a member of CTAs and in humans encodes a small protein containing 212 amino acids [17] which is expressed primarily in cells of trophoblastic lineage and exerts a fundamental role in placental function and development [18–21]. In spite of this placenta-specific expression pattern, there is a growing body of evidence showing that *PLAC1* is frequently activated and expressed in a wide variety of cancers including breast [22–26], lung [22–24, 27, 28], liver [23, 27, 29], colon [23, 24, 27, 30–32], stomach [22, 33, 34], ovary [22, 35, 36], uterus [37, 38], cervix [23, 39], and pancreas [40]. Recently, using tissue microarray, we showed the increased expression of PLAC1 in a stepwise manner from benign prostatic hyperplasia to prostate carcinoma, which expressed the highest levels of the molecule. In the majority of normal tissues, however, PLAC1 expression was not detectable [41].

There is limited data on the mechanistic role of PLAC1 in pathogenesis of cancer. Using knockdown experiments, Koslowski et al. showed that PLAC1 is involved in breast cancer cell proliferation, invasion and migration [22]. Based on preliminary data on its promoting function in cancer development and its expression in cancers of different histological origin, PLAC1 has been regarded as one of the potential CTAs for targeted cancer immunotherapy.

In this review, an overview will be provided on the PLAC1 molecule and current data summarized from our group as well as others with an emphasis on the importance of PLAC1 in cancer immunotherapy development.

PLAC1 molecule

Genomic organization of human and mouse *PLAC1/plac1* gene

In humans, *PLAC1* maps 65 kb telomeric to hypoxanthine–guanine phosphoribosyl transferase (*HPRT*) at Xq26. The mouse orthologue, *Plac1*, maps to the equivalent region of the mouse X chromosome [17]. The original study revealed that the human *PLAC1* gene had three exons in which the last exon is responsible for protein coding [17]. Later, Chen et al. showed that *PLAC1/plac1* of human and mouse origins contained six exons; with the last exon functioning as the protein-coding exon. A segment upstream to the first exon includes one active promoter (P1). A second promoter (P2) discovered earlier, is located just upstream to exon 4. Transcripts starting at P1 always include exons 5 and 6 and may include exons 1, 2 or 3 but always skip exon 4; whereas transcripts originating from P2 exclusively include exons 4, 5 and 6 (Fig. 1) [42].

Sequencing analysis of the mouse *plac1* and the human *PLAC1* genes revealed 75% and 60% identity at the nucleotide and amino acid levels, respectively [17]. Calculation of the ratio of nonsynonymous (dN) and synonymous (dS) mutation rates, dN/dS, to investigate the evolutionary selection of *PLAC1* among 25 species provided convincing evidence on purifying selection of *PLAC1* throughout the process of mammalian development [43]. This feature along with the divergent expression of PLAC1 in mammals of different species highlights the fundamental role of *PLAC1* in the developmental process. To find the potential origin of *PLAC1* gene, the consensus first 119 amino acids (out of 212) of human *PLAC1* nucleotide sequence was assembled in silico and used to examine the genomes of several non-placental and placental species. Results showed that the non-placental species lacked a sequence or sequence fragments that could be considered as ancestral *PLAC1*, while the results of such analysis suggested that *PLAC1* arose de novo in the placental mammalian genome. Based on phylogenic data, thoroughly analyzed by Devor, it seems that PLAC1 has emerged some 165 million years ago [44].

Regulatory mechanisms of *PLAC1/plac1* gene expression

Great efforts have been made to understand the regulatory mechanisms of *PLAC1* expression and in this regard, differential usage of P1 and P2 promoters in different tissues has been the subject of considerable amounts of research. P1 and P2 promoters are activated by retinoid X receptor alpha (RXRα) in conjunction with liver X receptor (LXRα or LXRβ) in both mice and humans [42]. Reverse Transcription-quantitative Polymerase Chain Reaction (RT-qPCR) and fluorescent reporter assay have revealed differential P1 and P2 promoter usage in placenta, tumors, and several cancer cell lines [37, 38, 42]. While in human placenta, the P2 promoter is preferentially utilized for the initiation of transcription, the P1 promoter is predominantly favored in tumors, cell lines, and human fetal tissues [38]. In human and mouse placenta, transcripts from both promoters can be traced, but transcripts from P2 were found to be eightfold and tenfold more abundant than those from P1 in human and mouse placenta, respectively. There is a great variability of promoter usage in human cancer cell lines. In choriocarcinoma cell lines BeWo, JEG-3, and JAR the P1 is preferentially used, while the breast cancer cell line MCF-7, uses the P2 [38, 42]. Similarly, the P1 promoter preferentially contributes to the transcription of *PLAC1* in all endometrial tumors, but in endometrial cancer cell lines, there is a mixture of P1-driven and P2-driven transcripts [37].

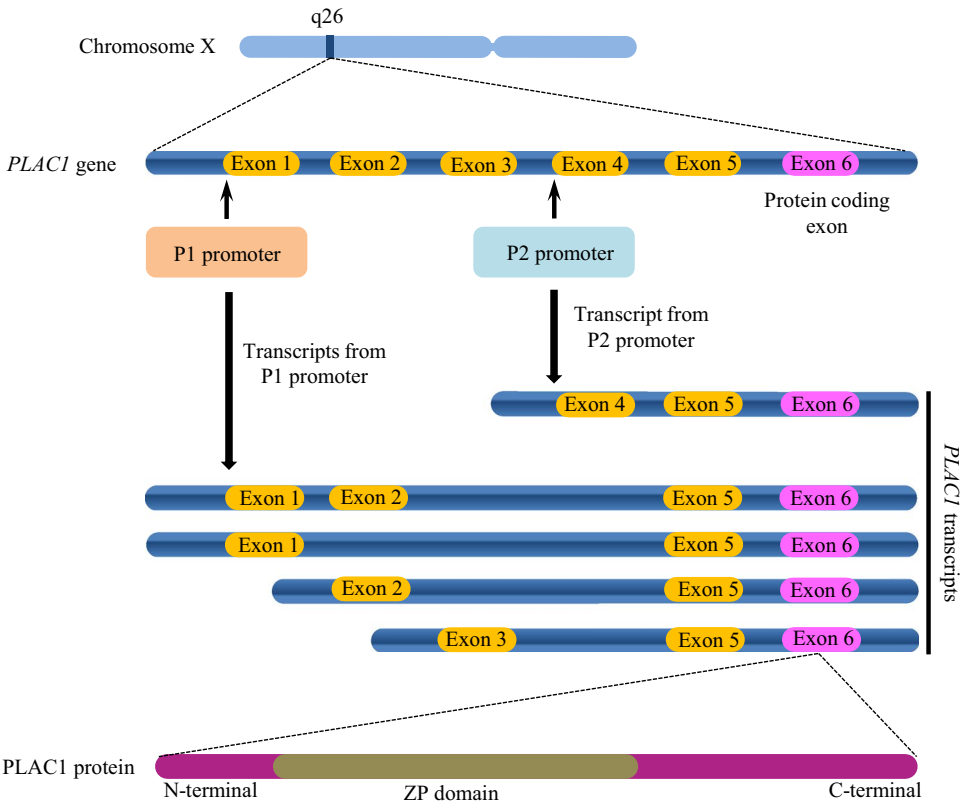


Fig. 1 The genomic organization, transcripts and protein structure of human PLAC1. *PLAC1* gene is located on chromosome Xq26 and contains six exons and two promoters, P1 and P2. P1 and P2 are located just upstream to exons 1 and 4, respectively. Transcripts initiating at P1 always include exons 5 and 6 and may include exons 1, 2 or 3 but always skip exon 4; however, transcripts starting from P2 exclusively include exons 4, 5 and 6. The last exon (exon 6) encodes a putative protein consisting of 212 amino acids. PLAC1 protein has a large extracellular portion containing a truncated zona pellucida (ZP) domain that ranges from amino acids 29–116

The rationale behind the differential usage of P1 vs. P2 by different cells is not clear at present. It is well established that a gene transcript originating from different promoters shows different stabilities [45]. Such phenomena may reflect the differential utilization of *PLAC1* promoters. Indeed, the aforesaid data indicate that besides endogenous factors, epigenetic factors are also involved in the regulation of *PLAC1* promoter usage. In particular, different transcription factors available at different microenvironments, the extent of promoter hypermethylation, responsiveness to steroid hormones as well as the ease for transcription from a certain promoter could also be regarded as the basis for *PLAC1* promoter switching in different organs and cells.

Chen et al. [46] showed that the expression of *PLAC1* was activated in SV40-transformed primary cell lines. Further transient transfection experiments with large T antigen (LT) of SV40 showed that the human tumor protein p53 (Tp53) and the retinoblastoma protein (RB) regulate *PLAC1* P1 promoter activity. In the presence of LT, endogenous Tp53 forms a stable complex with LT; hindering Tp53 to occupy *PLAC1* P1 promoter leading to *PLAC1* gene activation. Regarding the regulatory role of RB, *PLAC1* P1 promoter was found to be activated when RB was transfected into BeWo cells. Tp53 occupies P1 promoter, whereas RB shows no direct interaction with the P1 promotor; RB acts in correlation with the nuclear receptor LXR and co-activator NCOA2. Chen et al. also showed that *PLAC1* P1 promoter was normally enriched by histone 3 lysine 9 (H3K9) and histone 3 lysine 27 (H3K27) tri-methyl modifications in IMR-90 and WI38 cells, consistent with a repressed promoter status. Upon SV40 early region transfection to IMR-90 and WI38 cells, the promoter was depleted from these methylations, as expected for transcriptional activation. However, no significant changes in histone acetylation status were observed [46]. In spite of this as well as the findings showing that several germ cell and trophoblast-specific genes in cancer cells were activated in the presence of demethylating agents such as 5-aza-2′-deoxycytidine [6, 47–50], it was reported that *PLAC1* expression was not induced by 5-aza-2′-deoxycytidine treatment [22]. To explain this discrepancy, Chen et al. [46] found that *PLAC1* gene was suppressed in the presence of Tp53, although demethylation of the promoter occurred following SV40 early region transfection.

Notably, several cancer cell lines that express *PLAC1* carry mutations in Tp53. There are, however, examples of cell lines that carry Tp53 mutation(s) but express very low levels of *PLAC1* (for example, MOLT4 and U251) [23]. Not all mutations in Tp53 might affect its binding to *PLAC1* P1 promoter and consequently lead to activation of *PLAC1* in cancer cells. For instance, in serous ovarian and papillomavirus 16/18-positive cervical tumors, the wild-type Tp53 is associated with *PLAC1* transcription repression, whereas the mutant Tp53 protein activates *PLAC1* transcription that is particularly initiated from P1 promoter

[36, 39]. In this regard, more investigations are required to explore the nature of the mutations in Tp53 that have an impact on its repressor activity on *PLAC1* P1 promoter.

Transcription factors, CCAAT/enhancer-binding protein β-2 (C/EBPβ-2) and specificity protein 1 (Sp1) are required for activation of the *PLAC1* P2 promoter in SK-BR-3, MCF-7, and MDA-MB-231 breast cancer cell lines [51]. Using RT-qPCR and Western blot (WB) analyses, it was found that estrogen receptor alpha (ERα)-positive tumors and ERα-positive MCF-7 breast cancer cells express relatively significantly higher levels of *PLAC1* than ERα-negative tumors and ERα-negative MCF-7 breast cancer cells, respectively. In detail, it was observed that ERα-bound 17β-estradiol could activate *PLAC1* expression from the P2 promoter by the tethering of ERα to DNA-bound C/EBPβ-2, SP1, and NCOA3 [51, 52]. Upon 17β-estradiol treatment, specific employment of histone acetyl transferases p300, P300/CBP-associated factor (PCAF), general transcription factor IIB (TFIIB), and RNA polymerase II (Pol II) to the *PLAC1* P2 promoter was observed [52]. Later, Nagpal et al. demonstrated that estrogen induces miR (microRNA)-191 expression through dynamic binding of ERs to the estrogen response element (ERE) in the promoter of miR-191. Additionally, hypoxia and serum starvation induce miR-191 expression in MCF7 (ER-α+/β−) cells. Overexpression of miR-191 resulted in downregulation of special AT-rich sequence-binding protein-1 (SATB1) in MCF7 cells leading to *PLAC1* overexpression [53].

In a transgenic mouse model, *Plac1* transcription was upregulated by the expression of peroxisome proliferator-activated receptor-δ (PPARδ) through endogenous or synthetic PPARδ ligands in ER^+, progesterone receptor positive (PR^+), and $ErbB2^-$ mammary infiltrating ductal carcinoma [54]. PPARδ is a nuclear receptor which activates transcription of a variety of target genes by binding to the specific DNA elements. It has been shown that the C/EBPβ-driven transcription of *PLAC1* via P2 promotor is associated with PPARδ [54, 55]. Although the *PLAC1/Plac1* P1 promoter is upregulated by LXR and RXRα, the direct involvement of PPARδ with LXR and RXRα has not yet been investigated. Also, it was reported that peroxisome proliferator-activated receptor-γ (PPARγ) and vitamin D receptor had no detectable effects on *PLAC1/plac1* P1 promoter [42].

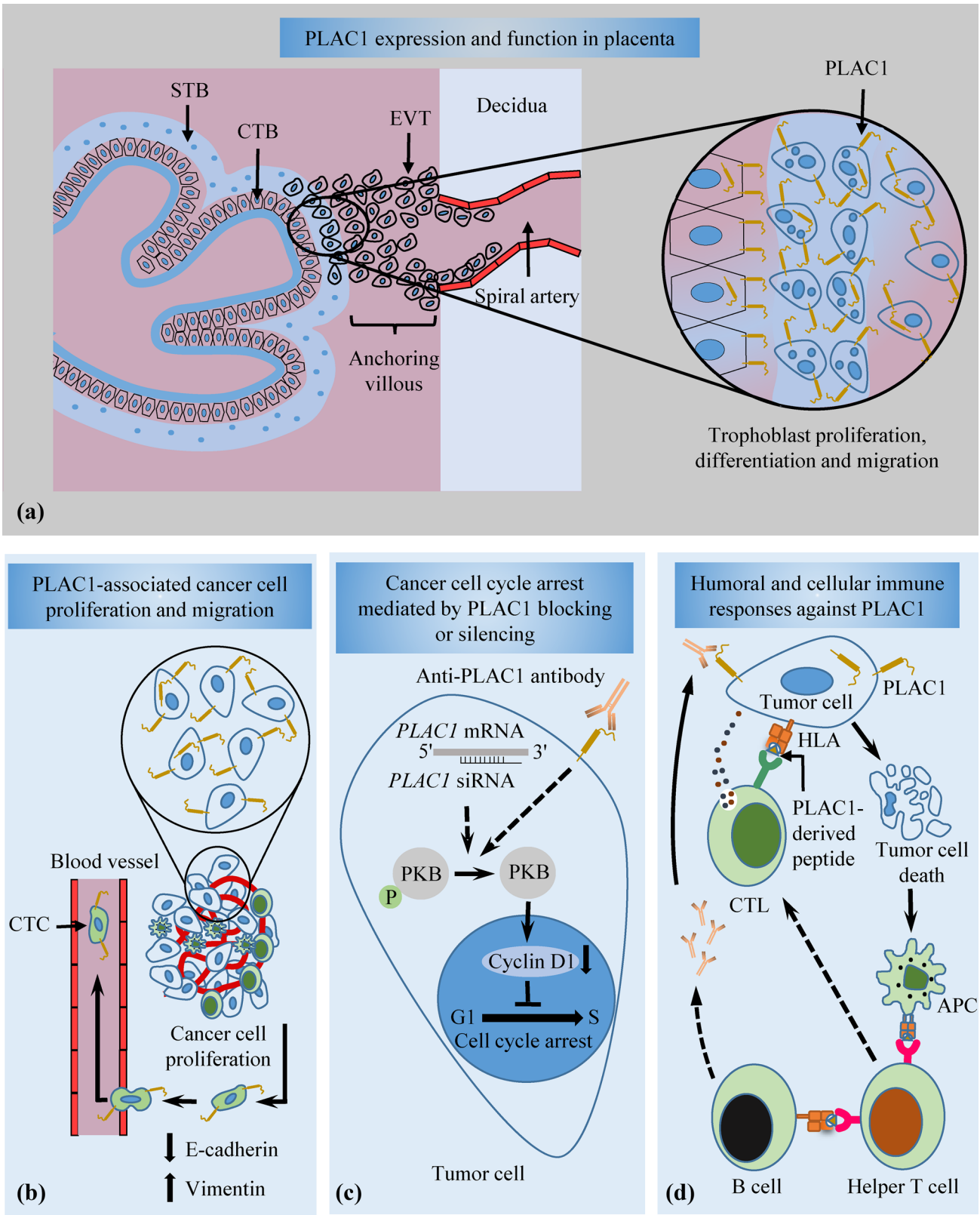
SRY-related HMG 2 box (SOX2) is another candidate gene for the regulation of *PLAC1*. SOX2 encodes a member of the SOX family of transcription factors which is expressed in normal gastric mucosa but is frequently downregulated in human gastric cancer tissues and cell lines [56, 57]. Otsubo et al. showed that *PLAC1* expression was significantly upregulated by SOX2 knocking down using miR-126 and small interfering RNA (siRNA) in a gastric cancer cell line, HSC43 [58].

The effect of growth factors in the regulation of *PLAC1* expression has also been investigated. Fant et al. showed that keratinocyte growth factor (KGF) stimulated PLAC1 expression in BeWo cells [59]. KGF stimulation was significantly inhibited by PD-98059 and wortmannin suggesting mediation of KGF effect through MAP kinase and PI-3 kinase-dependent signaling pathways [60], whereas, insulin-like growth factor II (IGF-II) [59] and epidermal growth factor (EGF) [60] were not able to modulate *PLAC1* expression. In another study, it was shown that RNA interference-mediated *PLAC1* silencing in MCF-7, BT-549, Bel-7402, and HepG2 significantly reduced cyclin D1 transcript and protein expression [22, 29]. This finding was in line with reports showing that activation of the *PLAC1* P2 promoter in SK-BR-3, MCF-7, and MDA-MB-231 breast cancer cells by C/EBPβ-2 resulted in selectively activation of cyclin D1 [51]. In addition, it was revealed that 17β-estradiol, that activates *PLAC1* expression through a non-classical pathway by P2 promoter, [51, 52] induced Cyclin D1 expression [61]. Cyclin D1 is a major regulator of the G1-S progression of the cell cycle. Cyclin D1 expression is controlled through a phosphatidylinositol 3-kinase (PI3K)/AKT-dependent pathway [62, 63]. Moreover, siRNA-mediated *PLAC1* silencing or PLAC1 blockade by anti-PLAC1 antibody (Ab) resulted in a reduction of phosphorylated protein kinase B (PKB), also known as Akt, levels [22, 28, 29]; suggesting that AKT kinase activation is involved in the execution of down-stream effects of PLAC1. Taken together, it may be concluded that *PLAC1* expression is significantly regulated by MAP kinase and PI-3 kinase-dependent signaling pathways. *PLAC1* mRNA level is increased during in vitro differentiation of cytotrophoblast (CTB) to syncytiotrophoblasts (STBs) [60, 64] indicating involvement of the same signaling pathways for *PLAC1* expression in the CTB differentiation process.

Most of the studies mentioned above have dealt with the regulation of PLAC1 gene expression. Whether or not an expression of PLAC1 at the protein level is also influenced in parallel by the aforesaid regulators, needs further investigations, although Koslowski et al. showed that PLAC1 protein levels in breast cancer specimens correlated well with its corresponding transcript levels [22].

PLAC1 protein structure and topology

The human *PLAC1* gene encodes a putative protein consisting of 212 amino acids, whereas the mouse *plac1* gene encodes a highly homologous but shorter product with 173 amino acids [17]. In silico analysis of membranous topology of the PLAC1 sequence predicted a type II membrane protein with a short N-terminal intracellular component, a single transmembrane helix spanning amino acids 5–22 and a large extracellular domain composed of amino acids 23–212. Amino acids 29–119 of the extracellular portion of



PLAC1 represent a truncated zona pellucida (ZP) domain (Fig. 1) [22]. Human PLAC1 contains four putative O-glycosylation sites and five potential phosphorylation sites [65]. Different molecular weights have been reported so far for PLAC1 including 24 KD [66], 25.6 KD [30], 26 KD [22], 27 KD [67], and 28–30 KD [65] which, besides technical

◂**Fig. 2** PLAC1 expression and function in placenta and cancer, PLAC1 is expressed by cytotrophoblasts (CTB), syncytiotrophoblasts (STB) and extravillous trophoblasts (EVT) and is associated with cell proliferation, differentiation and migration. PLAC1 is known to play a key role in placental development (**a**). PLAC1 is also re-expressed in several solid tumors and in most human cancer cell lines and promotes cancer cell proliferation. Expression of PLAC1 is also a triggering factor for enhanced migration of cancer cells that have undergone epithelial-mesenchymal transition (EMT) process through downregulation of E-cadherin and upregulation of vimentin (**b**). PLAC1 blocking by specific antibodies or silencing by RNA interference leads to cell cycle arrest in cancer cells through AKT and reduction of phosphorylated protein kinase B (PKB) and cyclin D1 levels which eventually leads to cell cycle arrest (**c**). PLAC1 expression in cancer cells induces cellular and humoral immune responses. Antigen-presenting cells (APC) endocytose cancer apoptotic bodies and present PLAC1-derived peptides to T cells which subsequently leads to induction of PLAC1-specific cytotoxic T cells (CTL) and antibody responses. CTLs kill PLAC1^{+} cancer cells by releasing cytotoxic lytic granules and anti-PLAC1 antibodies impair cancer cell proliferation and induce their apoptosis. It is, however, not clear to what extent anti-PLAC1 immune responses are effective in cancer control in vivo (**d**)

variations, can be attributed in part to the potential different post-translational modifications (PTM) in different cells. Although not yet specified, the presence of different isoforms cannot be excluded.

The ZP domain which is found in a variety of extracellular exposed receptor-like proteins, including transforming growth factor-β (TGF-β) receptor type III, uromodulin and glycoprotein GP2 [68], is a sequence of approximately 260 amino acids [69]. Based on the number of highly conserved cysteine residues, the ZP domain is classified into two classes, type I and II. Type I ZP domains such as ZP3 possess 8 highly conserved cysteine residues, whereas type II ZP domains (ZP1 and ZP2) have 10 conserved cysteine residues. In addition, the ZP domain consists of two subdomains: the N-terminal subdomain (ZP-N) and the C-terminal subdomain (ZP-C) [70]. The ZP domain of PLAC1 bears a significant degree of homology to the N-terminal subdomain of sperm receptor, ZP3 [17]. The ZP3-like region classifies PLAC1 as a cell adhesion molecule and suggests that it can mediate strong protein binding interactions [71]. Jovine et al. showed that *E. coli*-produced recombinant ZP3-N domain of mouse plac1 is able to fold independently and form homodimers consisting of two and four molecules of plac1 [71].

In an attempt to optimize the production of full-length human PLAC1 protein in prokaryotic systems, we overexpressed recombinant human PLAC1 using an expression matrix consisting of two expression vectors, five different *E. coli* hosts, and five solubilization conditions. Using Circular Dichroism (CD) we found that the secondary structure of PLAC1 consisted of 11.2% alpha-helix, 53% beta-sheets and turns, and 35.8% random structures. Ellman method also showed PLAC1 to contain four cysteine residues involved in disulfide bridges [72].

Phylogenic analyses of PLAC1 coding sequence and its amino acid translation in 54 placental mammalian species have represented thus far, twelve crown orders [44]. Moreover, 0–10 amino acid consistency scoring of PLAC1 among 25 species using PRofile ALIgNEment (PRALINE) [73, 74], a multiple sequence alignment application, showed that protein sequence conservation was highest in the 23 residues of the transmembrane domain (averaging 9.3 ± 1.2) and in the 63 residues of the ZP3 domain (averaging 8.7 ± 1.2) indicating that these regions of the molecule were functionally important. In contrast, the 23 residues of the signal peptide (averaging 7.0 ± 1.1) and the variable length C-terminal region (averaging 4.0 ± 2.7) were less conserved [43].

Expression profile of PLAC1

PLAC1 expression in normal cells and tissues

Most of the studies on PLAC1 expression in non-cancerous tissues of humans and mice at both gene and protein levels have been mainly focused on placenta. Cocchia et al. demonstrated that *Plac1* transcript was expressed in ectoplacental cone, giant cells, and labyrinthine trophoblasts of mouse placenta from 7.5 days post coitum (d.p.c.) to 14.5 d.p.c by in situ hybridization. On 14.5 d.p.c., when the mouse placenta was structurally complete, only a weak expression of *Plac1* was observed in the trophoblast giant cells [17]. This pattern is somehow different from human placenta in which a considerable expression of PLAC1 is still observed in the term syncytiotrophoblasts (authors' own observations). These findings were recently extended by Gu et al. who showed *plac1* expression in murine placental spongiotrophoblast layer and glycogen trophoblast cells [75]. Shi et al. [76] recently showed that plac1 protein was expressed in murine oocytes. Their finding suggests that plac1 is essential for oocyte meiosis and fertilization. Kong et al. showed that *Plac1* mRNA was localized to the hindbrain and lateral ventricles of wild-type embryos and its expression in murine fetal brain was remarkably decreased after birth. Moreover, *Plac1* transcript was found to be expressed in fetal tissues including lungs, kidney, intestine, liver, and heart. They also showed by immunohistochemical staining that Plac1 protein was localized to the apical surface of the epithelial cells lining, the developing airways of the lung and proximal renal tubules in mouse embryos [77].

In humans, *PLAC1*/PLAC1 expression is mainly restricted to placenta where high expression of this molecule is observed (Fig. 2a) [22–24, 27, 35, 66, 78]. Using high throughput transcript expression profiling systems such as Affymetrix and RNA-Seq, expression of *PLAC1* with relatively very low expression rank score in oocyte, adrenal tissue, testis, kidney, heart (https://bgee.org), dental pulp stem

cells, epidermal keratinocyte and eye cells (https://genevisible.com) has been predicted.

PLAC1 transcript is specifically expressed in trophoblast cells at all stages of gestation from weeks 8 to 41. No expression of *PLAC1* was observed in stromal compartment or maternal decidua [59].

Using Northern blot analysis, *PLAC1* mRNA was detected in placenta as a single 1.7 kb transcript [59], but no *PLAC1* transcript was observed in adult spleen, thymus, testis, prostate, ovary, small intestine, colon, peripheral blood leukocytes, liver, kidney, heart, brain, pancreas, and skeletal muscles [17, 59]. Contrary to this report, Wang et al. showed that *PLAC1* message was expressed in adult brain cerebellum [24]. There is a great inconsistency regarding *PLAC1* expression in human fetal tissues. In preliminary studies, no *PLAC1* expression was reported by Northern blot analysis in a variety of fetal tissues including fetal brain, liver, kidney, and lung [59]. The absence of *PLAC1* expression in fetal tissues as reported by Fant et al. could be attributed to the low sensitivity of the method they employed. A subsequent study using RT-qPCR amplification demonstrated that *PLAC1* was expressed in a variety of human fetal tissues including fetal brain, heart, liver, and kidney [38].

There is a very limited set of data regarding the expression of PLAC1 protein in human tissues. Except for placenta, there are some reports showing the expression of PLAC1 protein in human testis. Notably, Silva et al. [23] reported the expression of PLAC1 protein in human testis by IHC, a finding which was subsequently confirmed by a report of *PLAC1* transcript in human testis [24]. We and other researchers have shown that PLAC1 protein was localized to STB and villous CTBs by immunohistochemistry (IHC) and immunofluorescence (IF) staining [27, 60, 64–66, 78]. The circulating form of PLAC1 protein has not been detected in maternal serum, fetal cord blood serum and amniotic fluid by immunoblot experiments [65]. Although the expression of PLAC1 by proteomics approach has been predicted in esophagus, ovary and colonic epithelial cells (https://www.proteomicsdb.org), this needs to be confirmed.

Regarding a relatively fast growing interest observed in PLAC1 as a potential target in cancer immunotherapy, one main concern is whether the PLAC1 protein can be expressed in normal adult human tissues. In fact, there is a great paucity of data in this regard. The only available data is the absence of PLAC1 protein expression in normal human prostate [41, 66], endometrium, and lymph node [66]. Nonetheless, there is an urgent need for studies focusing on PLAC1 expression in human vital tissues.

PLAC1 expression in cancer cells and tissues

Expression of *PLAC1* has been extensively investigated in human cancer cell lines. Using RT-qPCR, Silva et al. showed that *PLAC1* was differentially expressed in a panel of 74 tumor cell lines. In this study, cell lines of breast, cervix, colon, liver, lung, and prostate cancers highly expressed *PLAC1* (> 10% of basal *PLAC1* expression in placenta), whereas melanoma, multiple myeloma, choriocarcinoma, and neuroblastoma cell lines showed low *PLAC1* expression (< 10% of basal *PLAC1* expression in placenta) [23]. *PLAC1* transcript was also reported to be expressed in 22 of 40 tumor cell lines from different origins [22]. Among five hepatocellular carcinoma (HCC) cell lines, BEL-7402, BEL-7405, SMMC-7721, HepG2, and HLE, *PLAC1* transcript was expressed in BEL-7402, SMMC-7721, HepG2, and HLE. In the same study, expression of *PLAC1* in LX-1 (lung), G1-112 (colon) and CX-1 (colon) was also reported [27]. *PLAC1* expression in breast cancer cell lines, MCF7 and MDA-MB-231 has also been reported [79]. Notably, Wang et al. showed that *PLAC1* was highly expressed in MDA-MB-231, a highly aggressive and invasive breast cancer cell line, whereas relatively lower expression was observed in the less aggressive breast tumor line, MD-MB-361 [24]. From 19 colorectal cancer cell lines, 31% showed positive *PLAC1* expression [31]. Recently, Yang et al. showed that *PLAC1* was expressed in several lung cancer cell lines including PC-9, SPC-A1, H1299, A549, H1650, H520, and SK-MES-1 [28]. Reportedly, EBV transformation affects *PLAC1* expression. Wang et al. showed that *PLAC1* was expressed approximately 6.5-fold higher in EBV positive compared to EBV negative Burkitt's lymphoma cell line MutuI [24].

Only a few reports on the PLAC1 expression at the protein level exist so far. Using WB, the expression of PLAC1 was found to be positive in HCC cell lines, HLE, Bel-7402, SMMC-7721, HepG2, and Huh7 [29]. Using WB and flow cytometry, we recently showed the expression of PLAC1 in prostate cancer cell lines, LNCaP, DU145, and PC3 [67]. We also showed PLAC1 to be expressed in LNCaP, Caov4, MCF7, MDA-MB-231, T47D, PC3, and SKOV-3 cancer cell lines using immunocytochemistry (ICC) [66]. Expression of PLAC1 in colorectal cancer cell lines is, to a great extent, cell-line-dependent. For instance, SW480 [30] and LS180 [67] cells did not express PLAC1, while SW620 showed a positive PLAC1 expression using ICC [30]. As with other TAA molecules, these data suggest a great variability of PLAC1 expression even in cancer cells from the histologically same origin and indicate that heterogeneity of cancer cells influences PLAC1 expression. Highly invasive SW620 colorectal cancer cells are $PLAC1^{+}$, while SW480 cells with lower invasive capacity do not express PLAC1 indicating a potential correlation between PLAC1 expression and the metastatic capacity of cancer cells. The same feature is also observed in the case of highly metastatic MDA-MB-231 and less invasive SK-BR-3 breast cancer cells (authors' unpublished data). Indeed, the variability of PLAC1 expression

level in different cancer cell lines ranges from few percent to as much as 95% of the cells (authors' personal data). We have also shown that the expression of PLAC1 in a given cell line may vary depending on the culture condition. This feature warrants further investigation on whether or not expression of PLAC1 is influenced by tumor environmental factors such as cell density, pH and oxygen tension.

PLAC1/PLAC1 expression in human tumor tissues has been extensively investigated. Although in some reports, the expression frequency of PLAC1 for the same tumor type is relatively consistent, a great heterogenicity exists in other cases. This variability could be attributed to different confounding factors: different tumor grades, number of samples and the most important factor: technical issues such as the using different primer sequences by different groups and the type of Abs which can affect the sensitivity and specificity of detection. So far, the expression of PLAC1 transcript and protein across different cancer types has been reported including breast [22, 25, 26], liver [27, 29], lung [22, 23, 28], gastric [33, 34], colorectal [27, 30–32], ovarian [35, 36], endometrium [37], cervix [39], prostate [41], and pancreas [40] cancers (Table 1). Based on the available reports, cancer tissues of breast [22], ovary [35, 36], endometrium [37], cervix [39], and prostate [41] are among the tissues with the highest *PALC1*/PLAC1 expression. Notably, the P2 promoter of PLAC1 is estrogen responsive [51], indicating that P2-driven PLAC1 expression is expectable in such hormone-sensitive tumors. It would be of great interest to test the extent of PLAC1 expression in testicular cancers as well. Based on the information presented above, it might be safe to assume that PLAC1 could be regarded as a potential cancer immunotherapy target for cancers of urogenital origin.

The presence of *PLAC1*/PLAC1 molecules in patient sera has also been investigated as a non-invasive screening tool for the detection of cancer. In this regard, Guo et al. investigated the expression of *PLAC1* along with nine other CTAs to detect circulating tumor cells (CTCs) in peripheral blood mononuclear cell (PBMC) fraction of 51 HCC patients using RT-qPCR. The overall positive detection rate for the 10 tested CTAs in general and *PLAC1,* in particular, were 70.6% and 19.6% (10/51) of patients, respectively [80]. Also, the presence of PLAC1 protein in preoperative/pretreatment breast cancer sera was analyzed. It was shown that the mean plus one standard deviation (mean + SD) of serum PLAC1 level exceeded in 67% of subjects with ductal carcinoma in situ (DCIS), 67% of HER2-positive, 73% of triple-negative, and 73% of estrogen-positive/progesterone-positive (ER^+/PR^+) breast cancer cells [25].

Based on its expression pattern in testis and in a wide variety of cancer tissues, Silva et al. [23] were the first to designate PLAC1 as cancer testis 92 (CT92). Although CTAs are basically classified as X chromosome-linked CTAs (X-CTA) and those that are encoded by non-X chromosomes (Non-X CTA) [81], there is a more practical classification [82] in which CTAs are divided into three categories based on their expression profiles: testis-restricted, testis/brain-restricted and testis-selective. Transcripts of testis-restricted CTAs are present only in adult testis and no other normal adult tissues, except for placenta, while testis/brain-restricted CTAs have additional expression profile in other adult immunorestricted sites (all regions of the brain). Testis-selective CTAs are expressed at a higher ratio in testis and placenta relative to normal tissues. Hofmann et al. showed that more than half of the CTAs are of testis-selective type, while 39 out of 153 CTAs belonged to the testis-selective category. Interestingly, most testis/placenta restricted CTAs are X-CTA indicating that this type of CTAs are under tight transcriptional control in somatic cells and reinforce their potential usefulness for therapeutic purposes.

Notably, expression of X-CTAs including MAGE-A3, MAGE-A4, MAGE-C1, NY-ESO-1, and GAGE proteins have also been documented in placental tissues. Accordingly, MAGE-A3 and MAGE-A4 are expressed by cytotrophoblasts and syncytiotrophoblasts in most periods of human gestation, while expression of NY-ESO-1 and GAGE is mostly restricted to the first trimester [83] indicating that expression of CTAs in placenta is spatio-temporally regulated. This feature highlights the differential function of different CTAs during placental development.

These findings imply that most CTAs are expressed in placenta. Although the term "cancer testis" describes the specific pattern of expression of most of the CTAs, it does not specifically cover the majority of the CTAs, like PLAC1, which are also expressed in placental trophoblsts. The fact that the expression of most CT genes is restricted to cancers, gametes and trophoblast cells, suggests a common mechanism operational in these cells. In this regard, we propose that the term "cancer–testis–placental antigens (CTP)" better describes such CT antigens as PLAC1 which, in addition to male germ cells and cancers, are also expressed by placental trophoblasts. Although the knowledge about the function of CTAs is still limited, there are reports implying the involvement of CTAs in regulating the basic cellular processes such as apoptosis [84], cell cycle [85], transcription [86], proliferation [87] and susceptibility to cytokine effects in tumor cells [88]. In this regard, it is conceivable to imagine that those CT antigens expressed in placenta (CTP antigens) exert the very same function in placenta.

PLAC1 protein localization in normal and cancer cells

To get insight into the extent to which PLAC1 could be considered as a potential target for antibody-mediated cancer immunotherapy, localization of the molecule at subcellular levels in both normal and cancer tissues has been

Table 1 PLAC1 expression in tumors and correlation with clinicopathological parameters

Cancer type	Detection system			PLAC1 positive (%)	Reported correlation with clinicopathological parameters	References
	PCR	WB	IHC			
Different cancer types	+			86/225 (38%)	PLAC1 expression did not correlate with histologic subtype, tumor stage or tumor grade	[22]
Breast	+			51/62 (82%)		
		+	+	NS		
			+	97%	NS	[25]
			+	NS	PLAC1 expression positively correlates with clinical stage, lymph node metastasis, hormone receptor status, and overall patient survival	[26]
Hepatocellular	+			22/69 (32%)	NS	[27]
			+	7/30 (23%)		
			+	25/46 (54.3%)	PLAC1 expression did not correlated with HBsAg positive results, serum alpha feto protein and cirrhosis PLAC1 expression significantly correlated with metastasis of HCC	[29]
Lung	+			21/50 (42%)	PLAC1 expression did not correlate with histologic subtype, tumor stage or tumor grade	[22]
	+			119/156 (76.3%)	NS	[23]
	+		+	NS	PLAC1 expression did not correlate with histologic subtype, sex and age Higher PLAC1 expression levels significantly correlated with patients' poor prognosis, shorter survival time, TNM stage, and lymph node metastasis PLAC1 protein levels were higher in the moderately- and poorly differentiated tumor tissues than those in the highly differentiated tumor tissues	[28]
Gastric	+			14/28 (50%)	NS	[33]
			+	73/119 (61.3%)	The overall survival of patients with PLAC1 expression was significantly lower than that of patients with PLAC1 negative tumors	[34]
Colorectal	+			22 of 42 (47.6%)	NS	[27]
	+		+	20 of 42 (47.6%) (PCR) 12 of 42 (28.6%) (IHC)	NS	[30]
	+			10/78 (12.8%)	PLAC1 expression significantly correlated with the overall stage	[31]
			+	55 of 95 (56.7%)	PLAC1expression in poorly differentiated colorectal primary adenocarcinomas was significantly higher than that in well differentiated and moderately differentiated adenocarcinomas Higher PLAC1 expression significantly correlated with TNM stage and lymph node metastasis	[32]
Ovary	+			21/101 (21%)	The presence of PLAC1 did not have a statistically significant effect on recurrence-free and overall survival	[35]
	+			38/38 (100%)	A significant negative relationship between *PLAC1* expression and patient survival exists in serous ovarian tumors	[36]
Endometrium	+			24/24 (100%)	PLAC1 expression was significantly higher in more advanced, more aggressive endometrial serous adenocarcinomas and carcinosarcomas than that in endometrioid adenocarcinomas	[37]
Cervix (papillomavirus (HPV) 16/18 positive)	+			16/16 (100%)	Highest levels of expression were found in the more aggressive squamous and adenosquamous histologic types compared with adenocarcinomas	[39]

Table 1 (continued)

Cancer type	Detection system			PLAC1 positive (%)	Reported correlation with clinicopathological parameters	References
	PCR	WB	IHC			
Prostate			+	154/154 (100%)	PLAC1 expression was positively associated with Gleason score. In high grade prostatic tumor specimens, PLAC1 expression negatively correlated with prostate specific antigen (PSA) expression	[41]
Pancreatic ductal adenocarcinoma			+	41/93 (44.1%)	The frequency of PLAC1 positive patients was higher in poorly differentiated tumor group than those in the well-moderately differentiated tumor group PLAC1 expression significantly associated with decreased overall survival PLAC1 expression found to be an independent prognostic biomarker in the perineural invasion positive subgroup	[40]

NS not specified

extensively studied. Subcellular fractionation of the whole placental tissue and PLAC1 tracing by WB showed that PLAC1 was localized to the microsomal fraction, suggesting a membranous location for the PLAC1 protein [65]. Consistently, IHC staining by a rabbit anti-PLAC1 Ab against amino acids 125–212 [23] and a monoclonal anti-PLAC1 Ab against amino acids 166–177 [66] showed that PLAC1 was expressed on the plasma membranes of STBs and CTBs. Knocking down of PLAC1, using siRNA, revealed membranous localization of PLAC1 in breast cancer cell lines, MCF-7 and BT-549, by IF staining [22]. In accordance with previous reports, using flow cytometry it was recently shown that PLAC1 was expressed on the cell surface of about 30% of prostate cancer cell lines LNCaP, DU145 and PC3 [67]. Additionally, it was shown that PLAC1 localized mainly on the plasma membrane of ovarian cancer cell line Caov-4, using ICC [66]. Surface expression of PLAC1 has also been reported in breast [22], colon [30], prostate [41], and liver [29] tumors by immunohistochemical methods. Nevertheless, due to the technical limitations, precise localization of proteins by immunohistochemical staining is difficult to achieve. In parallel, there are reports pointing to the PLAC1 localization to cytoplasmic compartment of cancer cells and tissues [29, 30, 34, 40, 66]. Nuclear expression of PLAC1 in colorectal adenocarcinoma tissues [32] and stomach adenocarcinoma [34] has also been reported (Tables 2, 3). Using flow cytometry and IHC in a panel of 18 cancer cell lines from histologically different origins, it was found that the membrane expression of PLAC1 is not a ubiquitous phenomenon and some cancer cell lines, despite having positive signal in Western blotting, do not express this marker on the cell surface (unpublished data). The reason behind the heterogeneous localization of PLAC1 in different cells is not clear, yet. Based on the amino acid sequence of PLAC1 which contains a conserved signal sequence and a transmembrane domain representing type II integral membrane protein (IMP) topology, membranous localization of PLAC1 through secretory pathways is predictable. Nonetheless, cytoplasmic and nuclear localization of PLAC1 may imply the existence of a set of PLAC1 isoforms which could be generated by alternative splicing or other PTMs. Indeed, more importantly, different localizations of PLAC1 in cancer cells may imply that PLAC1 expression machinery is differentially regulated and that PLAC1 may exert different functions in different cancer cells.

To sum up, it is safe to conclude that subcellular localization of PLAC1 is influenced to some extent by the origin of the target cell and the molecular machinery that is exploited for PLAC1 expression in different cell types.

PLAC1 function

PLAC1/plac1 function in placental development

Based on the expression profile of *PLAC1* which is primarily restricted to trophoblast lineage cells, Jackman et al. sought to determine if *Plac1* was necessary for placental and embryonic development by examining a mutant mouse model (Fig. 2a). They showed that plac1 disruption caused placental hyperplasia and intrauterine growth restriction [20]. This finding was consistent with a study demonstrating that mouse *Plac1* was downregulated in hyperplastic placenta caused by interspecies hybridization [19].

In contrast, *Plac1* was found to be one of the up-regulated genes in the hyperplastic placenta generated by nuclear transfer [18]. To explain this discrepancy, Muto et al. expressed *plac1* to cover the *Plac1* knockout placental dysfunction by lentiviral vector-mediated placenta-specific *Plac1* transgene expression. They observed that *Plac1* knocking out caused

Table 2 PLAC1 localization in human cancer cell lines

Cancer type	Cell line	Type of antibody	Immunogen	Staining technique	Reported localization	References
Breast	MCF-7 BT- 549	pAb	aa:117–127	IF	Cell membrane	[22]
	MCF-7 MDA-MB-231 T47D	mAb	aa:166–177	ICC	Mainly cytoplasmic with some degree of cell membrane distribution	[66]
Prostate	LNCap				Mainly cell membrane	
	PC3				Mainly cytoplasmic with some degree of cell membrane distribution	
Ovary	SKOV-3					
	Caov-4				Mainly cell membrane	
Colon	SW 620	pAb	Recombinant protein expressed in *E. coli* or synthetic peptides (p117–128 and p188–200)	ICC	Cytoplasmic	[30]

pAb polyclonal antibody, *mAb* monoclonal antibody, *IF* immunofluorescence, *ICC* immunocytochemistry

Table 3 PLAC1 localization in human cancer tissues using IHC

Cancer type	Type of antibody	Source of antibody	Immunogen	Reported localization	References
Breast	pAb	Squarix	117–127	Cell membrane	[22]
Colon	pAb	Home-made	Recombinant protein expressed in *E. coli* or synthetic peptides (p117–128 and p188–200)	Cytoplasmic and cell membrane	[30]
Colorectal carcinoma	pAb	Gifted by professor Michael Fant	NS	Nuclear and cytoplasmic (based on differentiation state)	[32]
Prostate	pAb	Home-made	166–177	Cytoplasmic and cell membrane	[41]
Pancreatic ductal adenocarcinoma	pAb	Abcam	NS	Cytoplasmic	[40]
Primary gastric adenocarcinoma	pAb	NS	NS	Nuclear and cytoplasmic	[34]
Hepatocellular Carcinoma	NS	Abcam	NS	Cytoplasmic and cell membrane	[29]
	pAb	Home-made	Mixture of 117–128 and 188–200	NS (cytoplasmic based on the presented image)	[27]

pAb polyclonal antibody, *NS* not specified

placental hyperplasia with encroaching junctional zone, and morphological impairment of maternal blood sinuses in the labyrinth zone. Unexpectedly, *Plac1* transduction into *Plac1* knockout blastocysts did not normalize placental hyperplasia and encroachment of junctional zone into the labyrinth zone, while it caused improvement of the fetal development and the morphology of maternal blood sinuses in the labyrinth zone. Interestingly, *Plac1* transduction into the wild type placenta resulted in placental hyperplasia without the encroaching of the junctional zone. These results clearly showed that fine-tuning of *Plac1* expression level is needed for proper and normal placentation; hence deviation from this might result in abnormal placental growth and development. Noteworthy, this study also highlighted and reinforced the importance of fetal *Plac1* expression for normal placentation and function. The authors concluded that *Plac1* was involved in trophoblast cell proliferation, differentiation and migration [21]. Recently, using RT-qPCR Gu et al. showed that *Plac1* transcripts increased during trophoblast stem cell differentiation [75].

Using a mutant mouse model, Suzanne et al. [20] investigated the parental origin of *Plac1* expression. They

showed that placenta and embryos were severely affected when *Plac1*-null allele was inherited from the mother. At E16.5, knockout and heterozygous placenta that inherited Plac1-null allele from the mother (Xm-X) weighed approximately 100% more than wild type placenta, whereas paternally inherited null allele heterozygous placenta (XXp−) showed comparable growth to wild type placentas demonstrating paternal imprinting of *Plac1*. The same group also showed that XXp− *Plac1* heterozygotes did not show lethal hydrocephalus, while 20% of the surviving Xm-Y (knockout) males as well as 10–15% of the Xm-X females (mutant maternal allele) developed lethal hydrocephalus at 4–8 weeks [77] confirming paternal imprinting of *Plac1*. However, they also showed at both gene and protein levels that in Xm-X placentas, the level of *Plac1* expression was approximately 10–20% of WT expression, confirming partial escape of the paternal *Plac1* allele from total inactivation. This finding was supported by the experiments demonstrating that *Plac1* KO male mice could survive, but they showed decreased viability. In line with this finding, there are also documents showing that about 15% of X-linked genes are estimated to escape complete inactivation in the process of somatic X-inactivation [89]. In conclusion, it seems that paternal *plac1* is partially inactivated during development and contributes in part to normal placental and embryonic development. Muto et al. [21] also showed a similar pattern of paternal *plac1* allele inactivation after blastocyst implantation. Although they observed lower levels of *plac1* expression in male embryos than female embryos at E7, which is consistent with the concept of partial inactivation of paternal *Plac1*, this difference was not found to be statistically significant. Collectively, the prevailing theory in *Plac1* expression is partial imprinting of paternal *Plac1*.

Role of PLAC1 in cancer development and progression

It was demonstrated, using cell invasion and migration assays, in the trophoblast cell line HTR8/SVneo and primary extravillous trophoblasts (EVTs) that PLAC1 had an important role in EVTs invasion into the maternal decidua [78]. Moreover, plac1 has been judged to be among the genes associated with trophoblast invasion using a cDNA subtraction library between murine invasive and noninvasive trophoblasts [90]. These findings prompted researchers to investigate whether PLAC1 served a similar role in cancer.

Using siRNA silencing of *PLAC1*, Koslowski et al. showed that the motility, migration, and invasion of breast cancer cells, MCF-7 and BT-549, were profoundly dependent on *PLAC1* expression and that such silencing induced a G1-S cell cycle blockade with nearly complete abrogation of proliferation [22] (Fig. 2b, c). This group also confirmed their finding by inhibition of MCF-7 and BT-549 proliferating cells using blocking rabbit anti-PLAC1 Ab directed against amino acids 117–127 [22].

These findings were subsequently confirmed by another report in which knocking down of *PLAC1* by siRNA in hepatocellular carcinoma cells, Bel-7402 and HepG2, resulted in a substantial change in vital parameters including inhibition of tumor cell proliferation, increased apoptosis, induction of G1 cell cycle arrest, repressions of epithelial–mesenchymal transition (EMT), and decreased cell migration and invasion [29].

In line with previous reports, Yang et al. showed that silencing of *PLAC1* in non-small cell lung cancer cells (NSCLC) impaired cell proliferation, induced apoptosis, and impaired the invasive ability partially through the regulation of EMT-related proteins such as E-cadherin and vimentin [28].

The effect of PLAC1 in breast cancer cell invasion and metastasis was further investigated by Yongfei et al. [26]. They showed that PLAC1 interacted with Furin leading to Notch1 degradation and generation of Notch1 intracellular domain (NICD) fragments that could inhibit PTEN (phosphatase and tensin homolog) activity. PTEN is a known tumor suppressor protein and its inhibition promotes cell invasion and metastasis.

Besides the direct effect on cancer growth, a recent finding revealed that *Plac1* modulated a tolerogenic tumor microenvironment [91]. In this study, the authors showed that *Plac1* silencing considerably reduced mammary tumor growth in syngeneic mice but not in SCID mice suggesting that *Plac1* regulated adaptive immunity. Gene expression profiling of *Plac1* knockdown cells indicated reduction in several inflammatory mediators and chemokines including *CXCL1*. In this regard, it seems that the tumor-promoting effect of *Plac1* is mediated in part by modulating the chemokine axis. Collectively, it seems that the oncogenic function of PLAC1 is simultaneously exerted in parallel within tumor cells and in tumor microenvironment, a distinct feature that has not been reported for many TAAs. This aspect reinforces the potential usefulness of PLAC1 in targeted cancer immunotherapy, as PLAC1 targeting, besides therapeutic effects on cancer cells, might be able to restore the functionality of the immune cells in cancer microenvironment.

Correlation between PLAC1 expression in tumors and clinicopathological parameters

There are several reports investigating the potential correlation between PLAC1 expression and clinicopathological parameters of cancer patients. In none of the reports, a significant correlation was found between PLAC1 protein expression and age, sex, or tumor size [26, 29, 32, 34, 40]. Although there is some degree of inconsistency regarding

the correlation of PLAC1 expression and clinicopathological parameters in some types of cancer, the overall picture depicts poor prognosis and more aggressive nature of tumors with high PLAC1 expression (Table 1). In particular, PLAC1 expression levels significantly correlate with colorectal cancer stage [31], hepatocellular tumor metastasis [29], prostate cancer grade [41], pancreatic cancer differentiation [40], colorectal cancer differentiation, TNM stage and lymph node metastasis [32], lung cancer prognosis, survival time, TNM stage, lymph node metastasis, and differentiation [28], survival time in gastric [34], pancreatic [40], and ovarian cancer [36]. Furthermore, PLAC1 expression levels positively correlate with aggressiveness of endometrial serous adenocarcinomas [37] and cervix squamous cancers [39]. Nonetheless, Koslowski et al. reported that expression of PLAC1 did not correlate with histologic subtype, tumor stage or tumor grade in lung, gastric, and ovarian cancers [22]. Yuan et al. [25] demonstrated that no significant correlation was observed between serum PLAC1 levels with race, age at diagnosis, body mass index (BMI) or the presence of metastatic disease. Liu et al. reported that there was no significant relationship between PLAC1 expression in patients with gastric cancer and tumor position, differentiation, gross type, lymph node or TNM stage [34]. Tchabo et al. showed that the expression of PLAC1 in ovarian cancer did not have a significant effect on recurrence-free and overall survival [35]. In contrast, Devor et al. recently reported a significant negative relationship between *PLAC1* transcript and patient survival with serous ovarian tumor [36]. The different confounding factors listed above regarding expression of PLAC1 in human cancer tissues, sample size and sampling site may also be the case for discrepant results of the association studies mentioned above. Indeed, PLAC1 may exert different functions in different cancers. Moreover, the type of antibody used for IHC staining could also be considered as a potential confounding factor, as currently there is no standard antibody available for PLAC1 immunostaining. In our experience, we found great variability of IHC immunostaining patterns of the antibodies directed against different epitopes of PLAC1.

Therapeutic potential of PLAC1 targeting in cancer immunotherapy

Immune responses against PLAC1 in health and disease

The presence of humoral responses to PLAC1 in healthy individuals has been investigated by some researchers (Fig. 2d). Silva et al. reported that 4 of 78 (5%) healthy adults showed seropositivity with recombinant PLAC1 protein (amino acids 125–212) in enzyme-linked immunosorbent assay (ELISA). Notably, positive seroreactivity from healthy donors was restricted to females only indicating a possible sex-specific link with the immunogenicity of the antigen [23]. Whether or not the generation of antibodies to PLAC1 in the sera of a small subset of healthy women is a consequence of self-immunization following pregnancy warrants further investigation. There is a growing body of evidence showing that biological messages are continuously exported from placenta to maternal circulation through exosomes [92–95]. Indeed, apoptotic trophoblast cells are the main sources of fetal DNA and RNA in the maternal circulation [96, 97]. Maternal blood also contains *PLAC1* transcript, the level of which rapidly decreases after delivery [98–100]. In this regard, it is conceivable to imagine that at least a proportion of pregnant women became immunized against PLAC1 during pregnancy through the passenger syncytial aggregates or passenger trophoblasts. In such conditions as preeclampsia in which placental villi are damaged through the inadequate blood supply, the levels of *PLAC1*/PLAC1 mRNA/protein in maternal blood is substantially increased [99, 101–103].

Although the preliminary data show increased levels of anti-PLAC1 Ab in infertile women [104], mice immunized with PLAC1 peptides can give birth to normal pups [105]. Moreover, the aforesaid link between anti-PLAC1 Ab and infertility was not confirmed by other studies [106].

Restricted expression of *PLAC1* in normal tissues and its relatively high expression in cancer tissues have prompted the researchers to evaluate prognostic and diagnostic values of humoral and cellular immune responses against PLAC1 in a series of cancer patients. In one study using a peptide covering amino acids 125–212 from human PLAC1 as ELISA coating layer, 14 out of 226 plasma samples (6%) from NSCLC patients showed detectable levels of anti-PLAC1 antibodies [23]. Dong et al. analyzed the reactivity of sera from HCC patients with recombinant PLAC1 in WB analysis and reported that 4 out of 101 (3.8%) sera were reactive to PLAC1. By contrast, none of the serum samples from 50 healthy individuals were reactive to the recombinant PLAC1 in the same experiments [27].

To investigate the capacity of human PLAC1 to induce Ab responses in patients with colorectal cancer (CRC), serum samples from 42 CRC patients were surveyed by ELISA using whole recombinant PLAC1 protein as the ELISA coating layer. None of the 22 tumor samples negative for *PLAC1* mRNA showed seropositivity, while in 6 of the 20 patients with *PLAC1* mRNA positive tumors, anti-PLAC1 antibodies were detected. To link anti-PLAC1 antibodies with the tumor expression of PLAC1 protein, the authors observed that PLAC1 protein was expressed in 4 of the 6 seropositive patients. To interpret these inconsistent results, the authors explained that levels of PLAC1 protein were lower than the detection limit of their protein readout

systems (IHC and WB). Moreover, they observed that Ab responses were restricted to patients with a positive CD4 T cell response [30]. Kumara et al. [31] showed that none of the six colon cancer patients with positive *PLAC1* mRNA had anti-PLAC1 Ab in their sera when tested against full-length recombinant PLAC1. Similarly, none of the 20 epithelial ovarian tumor patients with positive *PLAC1* mRNA showed seroreactivity with recombinant PLAC1 protein in ELISA [35]. One limitation of these studies is the investigation of PLAC1 at the gene and not at the protein level. Expression of a gene does not necessarily lead to its protein expression.

In addition to humoral responses, cellular immune responses against PLAC1 in colorectal cancer patients have also been a matter of investigation (Fig. 2d). In one study, CD4 and CD8 T-cell responses were found in 58.3% (7/12) and 55.6% (10/18) of colon cancer patients with *PLAC1* mRNA positive tumors, respectively. Eighteen patients with positive results for *PLAC1* mRNA expression who had been tested for PLAC1-specific CD8 T-cell responses were followed for a mean of 27 months after their surgery. Interestingly, the majority of patients (90%, 9/10) with CD8 responses to the PLAC1 antigen survived during the follow-up period, whereas 6 of the 8 non-responders died during this period indicating a protective role of anti-PLAC1 cellular immune responses in colorectal cancer. This finding was further substantiated with Kaplan–Meier analysis indicating a significant correlation of anti-PLAC1 T-cell responses with the survival of the patients with CRC. However, neither significant differences in T cell proliferative response from PLAC1 responders and non-responders, nor correlations between positive T-cell responses and such clinicopathological parameters as tumor size, and TNM classification were observed [30].

In an attempt to cytotoxic T cell (CTL) epitope mapping of PLAC1, Liu et al. studied four predicted HLA-A*0201-restricted CTL epitopes of the molecule in T2 cell binding assay. They demonstrated that all predicted CTL epitopes [P8–16 (GLMILLTSA), P11–19 (ILLTSAPSA), P31–39 (SIDWFMVTV) and P58–66 (GLGCPPNHV)] bound to HLA-A*0201 with intermediate affinities. To assess their immunogenicity in vivo, CD8 T cells were generated from patients with colon cancer with positive PLAC1 mRNA. Generated CD8 T cells (as effector cells) were then assessed for IFNγ production after challenging with peptide-loaded T2 cells. CD8 T cells from the majority of patients responded to epitopes P31–39 (6/10) and P58–66 (7/11) but no reactivities to P8–16 and P11–19 were recorded [30]. They also showed that, $CD8^+$ T cells from three of five HLA-A*0201^+ CRC patients up-regulated Granzyme-B expression when co-cultured with HLA-A*0201^+/$PLAC1^+$ the colon cancer cell line SW620. No reaction was observed when PLAC1 negative SW480 colon cancer cells, with the same HLA haplotype, were co-cultured with CD8 clones suggesting specific response of the isolated $CD8^+$ T cells against PLAC1 [30]. This group extended their work in 11 HLA-A2 + CRC patients (9 PLAC1 $protein^+$ and 2 PLAC1 $protein^-$ tumors) using a new set of CTL epitopes. They used patients' own dendritic cells loaded with P41–50 (FMLNNDVCV) and P69–77 (HAYQFTYRV) peptides to prime specific CD8 cells. Stimulated CD8 cells were found to recognize T2 cells loaded with the corresponding peptides, released IFN-gamma and were able to specifically lyse HLA-$A2^+$/$PLAC1^+$ CRC cell line SW620. This response was not observed in PLAC1 negative CRC patients [32].

In another attempt to CTL epitope mapping of PLAC1, three HLA-A*0201-restricted epitopes were tested by T2 cell binding assay. CTLs of two of three HLA-A*0201^+ healthy donors primed by P28–36 (VLCSIDWFM) and P31–40 (SIDWFMVTV) peptides produced IFNγ in response to challenging with the peptide-loaded T2 cells. These IFNγ-producing CTLs could lyse HLA-A*0201^+/$PLAC1^+$ breast cancer cells MCF-7. They also showed, using human HLA transgenic mice, that P28–36 is a dominant CTL epitope naturally processed and presented in vivo and that CTLs against this peptide were able to lyse about one-third of MCF-7 cells. On the contrary, CTLs generated against P31–40 were functionally inactive [79]. Later, using the P28–36 peptide, PLAC1-specific TCR-engineered T cells were generated. In this study, the cDNAs encoding TCR chains specific for (HLA)-A*0201-restricted PLAC1 were cloned from CTLs which were formerly stimulated by autologous HLA-$A2^+$ dendritic cells loaded with a p28–36 peptide. They also showed that PLAC1-specific TCR-engineered T cells identified and eradicated $PLAC1^+$ breast cancer cell lines. Furthermore, using in vivo experiments, they demonstrated that $PLAC1^-$ specific TCR-engineered T cells significantly delayed tumor progression [107]. We observed that the majority of cancer cell lines, which do not express PLAC1 on their surfaces, express PLAC1 intracellularly (unpublished data). This aspect emphasizes the potential role of PLAC1-specific cell-mediated immune responses in cancer control.

These findings are in line with the newly emerging concept that PLAC1 can be viewed as a potential target for cancer immunotherapy and highlight the potential usefulness of harnessing anti-PLAC1 cell-mediated immune responses in controlling PLAC1 expressing tumors.

PLAC1 targeted therapy in cancer

There is a very limited data set on PLAC1 as a target for cancer immunotherapy. In a very recent report, we took the advantages of cancer specificity of anti-PLAC1 antibodies and cytotoxic agents for targeted therapy of prostate cancer. We showed that PLAC1 was rapidly internalized

following binding to anti-PLAC1 antibody, a process that was completed within an hour. Target specificity and internalization are among the most important characteristics of a successful antibody–drug conjugates [108–110] in which the Ab functions as a vehicle for intracellular delivery of cytotoxic agents [111, 112] to the tumor cells with minimal systemic toxicity and high therapeutic efficacy [108]. Based on the highly cytotoxic nature of antibody–drug conjugates (ADCs), high tumor selective expression and low/null normal tissue expression pattern of the molecular target is the most critical requirement for an ADC treatment [113], a feature that PLAC1 possesses. We observed that conjugation of the antineoplastic drug SN-38 to anti-PLAC1 Ab affected neither affinity nor the binding property of anti-PLAC1 Ab to the cell surface antigen. Interestingly, the ADC produced this way retained intrinsic Ab activity and showed selective cytotoxicity with enhanced IC50 about 15-fold lower than the free drug. The generated anti-PLAC1 ADC induced apoptosis in prostate cancer cell lines and in primary cultures of prostate cancer cells isolated from human tumors [67].

In 2009, National Cancer Institute proposed well-vetted periodization criteria for cancer antigens including, in descending order: (1) therapeutic function, (2) immunogenicity, (3) role of the antigen in oncogenicity, (4) specificity, (5) expression level and percent of antigen-positive cells, (6) stem cell expression, (7) number of patients with antigen-positive cancers, (8) number of antigenic epitopes and (9) cellular location of the antigen [2]. Accordingly, PLAC1 was ranked 47th in a list that comprised 75 cancer antigens. Notably, the therapeutic function of PLAC1 scored 0 because there was no report on the potential use of PLAC1 for cancer immunotherapy at that time. Based on our prior report on the therapeutic function of anti-PLAC1 Ab in prostate cancer, we believe that PLAC1 will now occupy a considerably higher rank in the list of cancer antigens. Moreover, for prostate cancer, PLAC1 is ubiquitously expressed by nearly all cancer cells in patients, a criterion which will definitely upgrade the periodization score of PLAC1.

Conclusions and therapeutic implications

Phonotypic and mechanistic similarities between cancer development and placentation have inspired researchers to find and introduce novel targets for cancer immunotherapy. PLAC1 is among that antigens that has gained the focus of many researchers since its first introduction in 2000. Up to now, the available literature reinforces a very restricted pattern of PLAC1 expression in normal cells and tissues, while the evidence on its aberrant expression in cancer tissues is steadily growing. Besides a fundamental role in placental development, there is growing evidence that PLAC1, expressed by many cancers of diverse origins, has a promoting effect in progression of several cancer types and is involved in cancer growth and invasion. Moreover, the protective humoral and cellular immune responses to PLAC1 are mounted in a proportion of cancer patients indicating that boosting such responses could be potentially beneficial for cancer treatment. These features potentiate the concept of PLAC1 as a molecular target for cancer immunotherapy and encourage further research exploiting the potential usefulness of anti-PLAC1 immune responses for cancer control. The stepwise increase in PLAC1 expression from prostatic benign hyperplasia to prostate cancers with high Gleason scores and the potential application of this molecule for antibody-mediated prostate cancer immunotherapy have been recently reported by our group [41, 67], the findings which await clinical studies to be consolidated. Nonetheless, studies on the immunobiology of PLAC1 in cancers are still limited and this molecule has not yet found the considerable place in the list of cancer antigens it deserves. In summary, cancer-targeted therapies directed against PLAC1 represent a new promise against several types of tumors. It should be emphasized, however, that based on the very heterogeneous nature of tumors and evasion mechanisms, it is unlikely that therapy solely against PLAC1 will be successful, and that combinational therapy targeting multiple TAAs, as well as modulation of the tumor microenvironment to be necessary.

Author contributions Jafar Mahmoudian, Roya Ghods, Mahboobeh Nazari and Amir-Hassan Zarnani drafted the manuscript. Jafar Mahmoudian and Amir-Hassan Zarnani designed the figures. Jafar Mahmoudian and Amir-Hassan Zarnani finalized the manuscript in consultation with Mahmood Jeddi-Tehrani, Mohammad Hossein Ghahremani and Seyed Nasser Ostad. Nassim Ghaffari-Tabrizi-Wizsy critically reviewed and edited the manuscript and made a substantial contribution in revising the manuscript according to the reviewers' comments. All authors checked and approved the final version of the manuscript.

Funding This work was partially supported by Grants from Tehran University of Medical Sciences (Grant no: 94-01-57-27939) and Avicenna Research Institute (Grant no: 960511-036).

Compliance with ethical standards

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

References

1. Hanahan D, Weinberg Robert A (2011) Hallmarks of cancer: the next generation. Cell 144(5):646–674. https://doi.org/10.1016/j.cell.2011.02.013

2. Cheever MA, Allison JP, Ferris AS, Finn OJ, Hastings BM, Hecht TT, Mellman I, Prindiville SA, Viner JL, Weiner LM, Matrisian LM (2009) The prioritization of cancer antigens: a national cancer institute pilot project for the acceleration of translational research. Clin Cancer Res Off J Am Assoc Cancer Res 15(17):5323–5337. https://doi.org/10.1158/1078-0432.CCR-09-0737
3. Ward JP, Gubin MM, Schreiber RD (2016) The role of neoantigens in naturally occurring and therapeutically induced immune responses to cancer. Adv Immunol 130:25–74. https://doi.org/10.1016/bs.ai.2016.01.001
4. Gubin MM, Artyomov MN, Mardis ER, Schreiber RD (2015) Tumor neoantigens: building a framework for personalized cancer immunotherapy. J Clin Investig 125(9):3413–3421. https://doi.org/10.1172/JCI80008
5. Coulie PG, Van den Eynde BJ, van der Bruggen P, Boon T (2014) Tumour antigens recognized by T lymphocytes: at the core of cancer immunotherapy. Nat Rev Cancer 14(2):135–146. https://doi.org/10.1038/nrc3670
6. Simpson AJ, Caballero OL, Jungbluth A, Chen YT, Old LJ (2005) Cancer/testis antigens, gametogenesis and cancer. Nat Rev Cancer 5(8):615–625. https://doi.org/10.1038/nrc1669
7. Yarchoan M, Johnson BA 3rd, Lutz ER, Laheru DA, Jaffee EM (2017) Targeting neoantigens to augment antitumour immunity. Nat Rev Cancer 17(4):209–222. https://doi.org/10.1038/nrc.2016.154
8. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, Powderly JD, Carvajal RD, Sosman JA, Atkins MB, Leming PD, Spigel DR, Antonia SJ, Horn L, Drake CG, Pardoll DM, Chen L, Sharfman WH, Anders RA, Taube JM, McMiller TL, Xu H, Korman AJ, Jure-Kunkel M, Agrawal S, McDonald D, Kollia GD, Gupta A, Wigginton JM, Sznol M (2012) Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med 366(26):2443–2454. https://doi.org/10.1056/NEJMoa1200690
9. Salmaninejad A, Zamani MR, Pourvahedi M, Golchehre Z, Hosseini Bereshneh A, Rezaei N (2016) Cancer/testis antigens: expression, regulation, tumor invasion, and use in immunotherapy of cancers. Immunol Investig 45(7):619–640. https://doi.org/10.1080/08820139.2016.1197241
10. Soundararajan R, Rao AJ (2004) Trophoblast 'pseudo-tumorigenesis': significance and contributory factors. Reprod Biol Endocrinol RBE 2:15. https://doi.org/10.1186/1477-7827-2-15
11. Wilczynski JR (2006) Cancer and pregnancy share similar mechanisms of immunological escape. Chemotherapy 52(3):107–110. https://doi.org/10.1159/000092537
12. Rama S, Suresh Y, Rao AJ (2001) Regulation of telomerase during human placental differentiation: a role for TGFbeta1. Mol Cell Endocrinol 182(2):233–248
13. Weier JF, Weier HU, Jung CJ, Gormley M, Zhou Y, Chu LW, Genbacev O, Wright AA, Fisher SJ (2005) Human cytotrophoblasts acquire aneuploidies as they differentiate to an invasive phenotype. Dev Biol 279(2):420–432. https://doi.org/10.1016/j.ydbio.2004.12.035
14. Chiu RW, Chim SS, Wong IH, Wong CS, Lee WS, To KF, Tong JH, Yuen RK, Shum AS, Chan JK, Chan LY, Yuen JW, Tong YK, Weier JF, Ferlatte C, Leung TN, Lau TK, Lo KW, Lo YM (2007) Hypermethylation of RASSF1A in human and rhesus placentas. Am J Pathol 170(3):941–950. https://doi.org/10.2353/ajpath.2007.060641
15. Moss RW (2008) The life and times of John Beard, DSc (1858–1924). Integr Cancer Ther 7(4):229–251. https://doi.org/10.1177/1534735408326174
16. Old LJ (2007) Cancer is a somatic cell pregnancy. Cancer Immun 7:19
17. Cocchia M, Huber R, Pantano S, Chen EY, Ma P, Forabosco A, Ko MS, Schlessinger D (2000) PLAC1, an Xq26 gene with placenta-specific expression. Genomics 68(3):305–312. https://doi.org/10.1006/geno.2000.6302
18. Suemizu H, Aiba K, Yoshikawa T, Sharov AA, Shimozawa N, Tamaoki N, Ko MS (2003) Expression profiling of placentomegaly associated with nuclear transplantation of mouse ES cells. Dev Biol 253(1):36–53
19. Singh U, Fohn LE, Wakayama T, Ohgane J, Steinhoff C, Lipkowitz B, Schulz R, Orth A, Ropers HH, Behringer RR, Tanaka S, Shiota K, Yanagimachi R, Nuber UA, Fundele R (2004) Different molecular mechanisms underlie placental overgrowth phenotypes caused by interspecies hybridization, cloning, and Esx1 mutation. Dev Dyn Off Publ Am Assoc Anat 230(1):149–164. https://doi.org/10.1002/dvdy.20024
20. Jackman SM, Kong X, Fant ME (2012) Plac1 (placenta-specific 1) is essential for normal placental and embryonic development. Mol Reprod Dev 79(8):564–572. https://doi.org/10.1002/mrd.22062
21. Muto M, Fujihara Y, Tobita T, Kiyozumi D, Ikawa M (2016) Lentiviral vector-mediated complementation restored fetal viability but not placental hyperplasia in Plac1-deficient mice. Biol Reprod 94(1):6. https://doi.org/10.1095/biolreprod.115.133454
22. Koslowski M, Sahin U, Mitnacht-Kraus R, Seitz G, Huber C, Tureci O (2007) A placenta-specific gene ectopically activated in many human cancers is essentially involved in malignant cell processes. Can Res 67(19):9528–9534. https://doi.org/10.1158/0008-5472.CAN-07-1350
23. Silva WA Jr, Gnjatic S, Ritter E, Chua R, Cohen T, Hsu M, Jungbluth AA, Altorki NK, Chen YT, Old LJ, Simpson AJ, Caballero OL (2007) PLAC1, a trophoblast-specific cell surface protein, is expressed in a range of human tumors and elicits spontaneous antibody responses. Cancer Immun 7:18
24. Wang X, Baddoo MC, Yin Q (2014) The placental specific gene, PLAC1, is induced by the Epstein–Barr virus and is expressed in human tumor cells. Virol J 11:107. https://doi.org/10.1186/1743-422X-11-107
25. Yuan H, Chen V, Boisvert M, Isaacs C, Glazer RI (2018) PLAC1 as a serum biomarker for breast cancer. PLoS One 13(2):e0192106. https://doi.org/10.1371/journal.pone.0192106
26. Li Y, Chu J, Li J, Feng W, Yang F, Wang Y, Zhang Y, Sun C, Yang M, Vasilatos SN, Huang Y, Fu Z, Yin Y (2018) Cancer/testis antigen-Plac1 promotes invasion and metastasis of breast cancer through Furin/NICD/PTEN signaling pathway. Mol Oncol. https://doi.org/10.1002/1878-0261.12311
27. Dong XY, Peng JR, Ye YJ, Chen HS, Zhang LJ, Pang XW, Li Y, Zhang Y, Wang S, Fant ME, Yin YH, Chen WF (2008) Plac1 is a tumor-specific antigen capable of eliciting spontaneous antibody responses in human cancer patients. Int J Cancer 122(9):2038–2043. https://doi.org/10.1002/ijc.23341
28. Yang L, Zha TQ, He X, Chen L, Zhu Q, Wu WB, Nie FQ, Wang Q, Zang CS, Zhang ML, He J, Li W, Jiang W, Lu KH (2018) Placenta-specific protein 1 promotes cell proliferation and invasion in non-small cell lung cancer. Oncol Rep 39(1):53–60. https://doi.org/10.3892/or.2017.6086
29. Wu Y, Lin X, Di X, Chen Y, Zhao H, Wang X (2017) Oncogenic function of Plac1 on the proliferation and metastasis in hepatocellular carcinoma cells. Oncol Rep 37(1):465–473. https://doi.org/10.3892/or.2016.5272
30. Liu FF, Dong XY, Pang XW, Xing Q, Wang HC, Zhang HG, Li Y, Yin YH, Fant M, Ye YJ, Shen DH, Zhang Y, Wang S, Chen WF (2008) The specific immune response to tumor antigen CP1 and its correlation with improved survival in colon cancer patients. Gastroenterology 134(4):998–1006. https://doi.org/10.1053/j.gastro.2008.01.029

31. Shantha Kumara HM, Grieco MJ, Caballero OL, Su T, Ahmed A, Ritter E, Gnjatic S, Cekic V, Old LJ, Simpson AJ, Cordon-Cardo C, Whelan RL (2012) MAGE-A3 is highly expressed in a subset of colorectal cancer patients. Cancer Immun 12:16
32. Liu F, Zhang H, Shen D, Wang S, Ye Y, Chen H, Pang X, Song Q, He P (2013) Identification of two new HLA-A*0201-restricted cytotoxic T lymphocyte epitopes from colorectal carcinoma-associated antigen PLAC1/CP1. J Gastroenterol 49(3):419–426. https://doi.org/10.1007/s00535-013-0811-4
33. Chen J, Pang XW, Liu FF, Dong XY, Wang HC, Wang S, Zhang Y, Chen WF (2006) PLAC1/CP1 gene expression and autologous humoral immunity in gastric cancer patients. Beijing da xue xue bao Yi xue ban (Journal of Peking University Health Sciences) 38(2):124–127
34. Liu F, Shen D, Kang X, Zhang C, Song Q (2015) New tumour antigen PLAC1/CP1, a potentially useful prognostic marker and immunotherapy target for gastric adenocarcinoma. J Clin Pathol 68(11):913–916. https://doi.org/10.1136/jclinpath-2015-202978
35. Tchabo NE, Mhawech-Fauceglia P, Caballero OL, Villella J, Beck AF, Miliotto AJ, Liao J, Andrews C, Lele S, Old LJ, Odunsi K (2009) Expression and serum immunoreactivity of developmentally restricted differentiation antigens in epithelial ovarian cancer. Cancer Immun 9:6
36. Devor EJ, Gonzalez-Bosquet J, Warrier A, Reyes HD, Ibik NV, Schickling BM, Newtson A, Goodheart MJ, Leslie KK (2017) p53 mutation status is a primary determinant of placenta-specific protein 1 expression in serous ovarian cancers. Int J Oncol. https://doi.org/10.3892/ijo.2017.3931
37. Devor EJ, Leslie KK (2013) The oncoplacental gene placenta-specific protein 1 is highly expressed in endometrial tumors and cell lines. Obstet Gynecol Int 2013:807849. https://doi.org/10.1155/2013/807849
38. Devor EJ, Reyes HD, Santillan DA, Santillan MK, Onukwugha C, Goodheart MJ, Leslie KK (2014) Placenta-specific protein 1: a potential key to many oncofetal-placental OB/GYN research questions. Obstet Gynecol Int 2014:678984. https://doi.org/10.1155/2014/678984
39. Devor EJ, Reyes HD, Gonzalez-Bosquet J, Warrier A, Kenzie SA, Ibik NV, Miller MD, Schickling BM, Goodheart MJ, Thiel KW, Leslie KK (2017) Placenta-specific protein 1 expression in human papillomavirus 16/18-positive cervical cancers is associated with tumor histology. Int J Gynecol Cancer Off J Int Gynecol Cancer Soc. https://doi.org/10.1097/igc.0000000000000957
40. Yin Y, Zhu X, Huang S, Zheng J, Zhang M, Kong W, Chen Q, Zhang Y, Chen X, Lin K, Ouyang X (2017) Expression and clinical significance of placenta-specific 1 in pancreatic ductal adenocarcinoma. Tumour Biol J Int Soc Oncodev Biol Med 39(6):1010428317699131. https://doi.org/10.1177/1010428317699131
41. Ghods R, Ghahremani MH, Madjd Z, Asgari M, Abolhasani M, Tavasoli S, Mahmoudi AR, Darzi M, Pasalar P, Jeddi-Tehrani M, Zarnani AH (2014) High placenta-specific 1/low prostate-specific antigen expression pattern in high-grade prostate adenocarcinoma. Cancer Immunol Immunother CII 63(12):1319–1327. https://doi.org/10.1007/s00262-014-1594-z
42. Chen Y, Moradin A, Schlessinger D, Nagaraja R (2011) RXRalpha and LXR activate two promoters in placenta- and tumor-specific expression of PLAC1. Placenta 32(11):877–884. https://doi.org/10.1016/j.placenta.2011.08.011
43. Devor EJ (2014) Placenta-specific protein 1 is conserved throughout the placentalia under purifying selection. Sci World J 2014:537356. https://doi.org/10.1155/2014/537356
44. Devor EJ (2016) Placenta-specific protein 1 (PLAC1) is a unique onco-fetal-placental protein and an underappreciated therapeutic target in cancer. Integr Cancer Sci Ther 3(3):479–483
45. Carlson M, Botstein D (1982) Two differentially regulated mRNAs with different 5′ ends encode secreted with intracellular forms of yeast invertase. Cell 28(1):145–154
46. Chen Y, Schlessinger D, Nagaraja R (2013) T antigen transformation reveals Tp53/RB-dependent route to PLAC1 transcription activation in primary fibroblasts. Oncogenesis 2:e67. https://doi.org/10.1038/oncsis.2013.31
47. De Smet C, De Backer O, Faraoni I, Lurquin C, Brasseur F, Boon T (1996) The activation of human gene MAGE-1 in tumor cells is correlated with genome-wide demethylation. Proc Natl Acad Sci USA 93(14):7149–7153
48. De Smet C, Lurquin C, Lethe B, Martelange V, Boon T (1999) DNA methylation is the primary silencing mechanism for a set of germ line- and tumor-specific genes with a CpG-rich promoter. Mol Cell Biol 19(11):7327–7335
49. Honda T, Tamura G, Waki T, Kawata S, Terashima M, Nishizuka S, Motoyama T (2004) Demethylation of MAGE promoters during gastric cancer progression. Br J Cancer 90(4):838–843. https://doi.org/10.1038/sj.bjc.6601600
50. Koslowski M, Bell C, Seitz G, Lehr HA, Roemer K, Muntefering H, Huber C, Sahin U, Tureci O (2004) Frequent nonrandom activation of germ-line genes in human cancer. Can Res 64(17):5988–5993. https://doi.org/10.1158/0008-5472.CAN-04-1187
51. Koslowski M, Tureci O, Biesterfeld S, Seitz G, Huber C, Sahin U (2009) Selective activation of trophoblast-specific PLAC1 in breast cancer by CCAAT/enhancer-binding protein beta (C/EBP-beta) isoform 2. J Biol Chem 284(42):28607–28615. https://doi.org/10.1074/jbc.M109.031120
52. Wagner M, Koslowski M, Paret C, Schmidt M, Tureci O, Sahin U (2013) NCOA3 is a selective co-activator of estrogen receptor alpha-mediated transactivation of PLAC1 in MCF-7 breast cancer cells. BMC Cancer 13:570. https://doi.org/10.1186/1471-2407-13-570
53. Nagpal N, Ahmad HM, Molparia B, Kulshreshtha R (2013) MicroRNA-191, an estrogen-responsive microRNA, functions as an oncogenic regulator in human breast cancer. Carcinogenesis 34(8):1889–1899. https://doi.org/10.1093/carcin/bgt107
54. Yuan H, Lu J, Xiao J, Upadhyay G, Umans R, Kallakury B, Yin Y, Fant ME, Kopelovich L, Glazer RI (2013) PPARdelta induces estrogen receptor-positive mammary neoplasia through an inflammatory and metabolic phenotype linked to mTOR activation. Can Res 73(14):4349–4361. https://doi.org/10.1158/0008-5472.CAN-13-0322
55. Brunelli L, Cieslik KA, Alcorn JL, Vatta M, Baldini A (2007) Peroxisome proliferator-activated receptor-delta upregulates 14-3-3 epsilon in human endothelial cells via CCAAT/enhancer binding protein-beta. Circ Res 100(5):e59–e71. https://doi.org/10.1161/01.RES.0000260805.99076.22
56. Li XL, Eishi Y, Bai YQ, Sakai H, Akiyama Y, Tani M, Takizawa T, Koike M, Yuasa Y (2004) Expression of the SRY-related HMG box protein SOX2 in human gastric carcinoma. Int J Oncol 24(2):257–263
57. Otsubo T, Akiyama Y, Yanagihara K, Yuasa Y (2008) SOX2 is frequently downregulated in gastric cancers and inhibits cell growth through cell-cycle arrest and apoptosis. Br J Cancer 98(4):824–831. https://doi.org/10.1038/sj.bjc.6604193
58. Otsubo T, Akiyama Y, Hashimoto Y, Shimada S, Goto K, Yuasa Y (2011) MicroRNA-126 inhibits SOX2 expression and contributes to gastric carcinogenesis. PLoS One 6(1):e16617. https://doi.org/10.1371/journal.pone.0016617
59. Fant M, Weisoly DL, Cocchia M, Huber R, Khan S, Lunt T, Schlessinger D (2002) PLAC1, a trophoblast-specific gene, is expressed throughout pregnancy in the human placenta and modulated by keratinocyte growth factor. Mol Reprod Dev 63(4):430–436. https://doi.org/10.1002/mrd.10200

60. Massabbal E, Parveen S, Weisoly DL, Nelson DM, Smith SD, Fant M (2005) PLAC1 expression increases during trophoblast differentiation: evidence for regulatory interactions with the fibroblast growth factor-7 (FGF-7) axis. Mol Reprod Dev 71(3):299–304. https://doi.org/10.1002/mrd.20272
61. Safe S, Kim K (2008) Non-classical genomic estrogen receptor (ER)/specificity protein and ER/activating protein-1 signaling pathways. J Mol Endocrinol 41(5):263–275. https://doi.org/10.1677/JME-08-0103
62. Muise-Helmericks RC, Grimes HL, Bellacosa A, Malstrom SE, Tsichlis PN, Rosen N (1998) Cyclin D expression is controlled post-transcriptionally via a phosphatidylinositol 3-kinase/Akt-dependent pathway. J Biol Chem 273(45):29864–29872
63. D'Amico M, Hulit J, Amanatullah DF, Zafonte BT, Albanese C, Bouzahzah B, Fu M, Augenlicht LH, Donehower LA, Takemaru K, Moon RT, Davis R, Lisanti MP, Shtutman M, Zhurinsky J, Ben-Ze'ev A, Troussard AA, Dedhar S, Pestell RG (2000) The integrin-linked kinase regulates the cyclin D1 gene through glycogen synthase kinase 3beta and cAMP-responsive element-binding protein-dependent pathways. J Biol Chem 275(42):32649–32657. https://doi.org/10.1074/jbc.M000643200
64. Chang WL, Wang H, Cui L, Peng NN, Fan X, Xue LQ, Yang Q (2016) PLAC1 is involved in human trophoblast syncytialization. Reprod Biol 16(3):218–224. https://doi.org/10.1016/j.repbio.2016.07.001
65. Fant M, Barerra-Saldana H, Dubinsky W, Poindexter B, Bick R (2007) The PLAC1 protein localizes to membranous compartments in the apical region of the syncytiotrophoblast. Mol Reprod Dev 74(7):922–929. https://doi.org/10.1002/mrd.20673
66. Ghods R, Ghahremani MH, Darzi M, Mahmoudi AR, Yeganeh O, Bayat AA, Pasalar P, Jeddi-Tehrani M, Zarnani AH (2014) Immunohistochemical characterization of novel murine monoclonal antibodies against human placenta-specific 1. Biotechnol Appl Biochem 61(3):363–369. https://doi.org/10.1002/bab.1177
67. Nejadmoghaddam MR, Zarnani AH, Ghahremanzadeh R, Ghods R, Mahmoudian J, Yousefi M, Nazari M, Ghahremani MH, Abolhasani M, Anissian A, Mahmoudi M, Dinarvand R (2017) Placenta-specific1 (PLAC1) is a potential target for antibody–drug conjugate-based prostate cancer immunotherapy. Sci Rep 7(1):13373. https://doi.org/10.1038/s41598-017-13682-9
68. Bork P, Sander C (1992) A large domain common to sperm receptors (Zp2 and Zp3) and TGF-β type III receptor. FEBS Lett 300(3):237–240. https://doi.org/10.1016/0014-5793(92)80853-9
69. Jovine L, Darie CC, Litscher ES, Wassarman PM (2005) Zona pellucida domain proteins. Annu Rev Biochem 74:83–114. https://doi.org/10.1146/annurev.biochem.74.082803.133039
70. Jovine L, Qi H, Williams Z, Litscher ES, Wassarman PM (2004) A duplicated motif controls assembly of zona pellucida domain proteins. Proc Natl Acad Sci USA 101(16):5922–5927. https://doi.org/10.1073/pnas.0401600101
71. Jovine L, Janssen WG, Litscher ES, Wassarman PM (2006) The PLAC1-homology region of the ZP domain is sufficient for protein polymerisation. BMC Biochem 7:11. https://doi.org/10.1186/1471-2091-7-11
72. Nazari M, Zarnani AH, Ghods R, Emamzadeh R, Najafzadeh S, Minai-Tehrani A, Mahmoudian J, Yousefi M, Vafaei S, Massahi S, Nejadmoghaddam MR (2017) Optimized protocol for soluble prokaryotic expression, purification and structural analysis of human placenta specific-1 (PLAC1). Protein Expr Purif 133:139–151. https://doi.org/10.1016/j.pep.2017.03.011
73. Heringa J (1999) Two strategies for sequence comparison: profile-preprocessed and secondary structure-induced multiple alignment. Comput Chem 23(3–4):341–364. https://doi.org/10.1016/s0097-8485(99)00012-1
74. Simossis VA, Heringa J (2005) PRALINE: a multiple sequence alignment toolbox that integrates homology-extended and secondary structure information. Nucleic Acids Res 33(Web Server issue):W289–W294. https://doi.org/10.1093/nar/gki390
75. Gu Y, Wan J, Yao L, Peng NN, Chang WL (2017) Plac1 expression pattern at the mouse fetomaternal interface and involvement in trophoblast differentiation. Cell Physiol Biochem Int J Exp Cell Physiol Biochem Pharmacol 43(5):2001–2009. https://doi.org/10.1159/000484154
76. Shi LY, Ma Y, Zhu GY, Liu JW, Zhou CX, Chen LJ, Wang Y, Li RC, Yang ZX, Zhang D (2018) Placenta-specific 1 regulates oocyte meiosis and fertilization through furin. FASEB J Off Publ Fed Am Soc Exp Biol. https://doi.org/10.1096/fj.201700922rr
77. Kong X, Jackman SM, Fant ME (2013) Plac1 (placenta-specific 1) is widely expressed during fetal development and is associated with a lethal form of hydrocephalus. Birth Defects Res A 97(9):571–577. https://doi.org/10.1002/bdra.23171
78. Chang WL, Yang Q, Zhang H, Lin HY, Zhou Z, Lu X, Zhu C, Xue LQ, Wang H (2014) Role of placenta-specific protein 1 in trophoblast invasion and migration. Reproduction 148(4):343–352. https://doi.org/10.1530/REP-14-0052
79. Liu W, Zhai M, Wu Z, Qi Y, Wu Y, Dai C, Sun M, Li L, Gao Y (2011) Identification of a novel HLA-A2-restricted cytotoxic T lymphocyte epitope from cancer–testis antigen PLAC1 in breast cancer. Amino Acids 42(6):2257–2265. https://doi.org/10.1007/s00726-011-0966-3
80. Guo L, Xu D, Lu Y, Peng J, Jiang L (2017) Detection of circulating tumor cells by reverse transcriptionquantitative polymerase chain reaction and magnetic activated cell sorting in the peripheral blood of patients with hepatocellular carcinoma. Mol Med Rep 16(5):5894–5900. https://doi.org/10.3892/mmr.2017.7372
81. Fratta E, Coral S, Covre A, Parisi G, Colizzi F, Danielli R, Nicolay HJ, Sigalotti L, Maio M (2011) The biology of cancer testis antigens: putative function, regulation and therapeutic potential. Mol Oncol 5(2):164–182. https://doi.org/10.1016/j.molonc.2011.02.001
82. Hofmann O, Caballero OL, Stevenson BJ, Chen YT, Cohen T, Chua R, Maher CA, Panji S, Schaefer U, Kruger A, Lehvaslaiho M, Carninci P, Hayashizaki Y, Jongeneel CV, Simpson AJ, Old LJ, Hide W (2008) Genome-wide analysis of cancer/testis gene expression. Proc Natl Acad Sci USA 105(51):20422–20427. https://doi.org/10.1073/pnas.0810777105
83. Jungbluth AA, Silva WA Jr, Iversen K, Frosina D, Zaidi B, Coplan K, Eastlake-Wade SK, Castelli SB, Spagnoli GC, Old LJ, Vogel M (2007) Expression of cancer–testis (CT) antigens in placenta. Cancer Immun 7:15
84. Cilensek ZM, Yehiely F, Kular RK, Deiss LP (2002) A member of the GAGE family of tumor antigens is an anti-apoptotic gene that confers resistance to Fas/CD95/APO-1, Interferon-gamma, taxol and gamma-irradiation. Cancer Biol Ther 1(4):380–387
85. Nagao T, Higashitsuji H, Nonoguchi K, Sakurai T, Dawson S, Mayer RJ, Itoh K, Fujita J (2003) MAGE-A4 interacts with the liver oncoprotein gankyrin and suppresses its tumorigenic activity. J Biol Chem 278(12):10668–10674. https://doi.org/10.1074/jbc.M206104200
86. Laduron S, Deplus R, Zhou S, Kholmanskikh O, Godelaine D, De Smet C, Hayward SD, Fuks F, Boon T, De Plaen E (2004) MAGE-A1 interacts with adaptor SKIP and the deacetylase HDAC1 to repress transcription. Nucleic Acids Res 32(14):4340–4350. https://doi.org/10.1093/nar/gkh735
87. Duan Z, Duan Y, Lamendola DE, Yusuf RZ, Naeem R, Penson RT, Seiden MV (2003) Overexpression of MAGE/GAGE genes in paclitaxel/doxorubicin-resistant human cancer cell lines. Clin Cancer Res Off J Am Assoc Cancer Res 9(7):2778–2785

88. Park JH, Kong GH, Lee SW (2002) hMAGE-A1 overexpression reduces TNF-alpha cytotoxicity in ME-180 cells. Mol Cells 14(1):122–129
89. Yang F, Babak T, Shendure J, Disteche CM (2010) Global survey of escape from X inactivation by RNA-sequencing in mouse. Genome Res 20(5):614–622. https://doi.org/10.1101/gr.103200.109
90. Hemberger M, Himmelbauer H, Ruschmann J, Zeitz C, Fundele R (2000) cDNA subtraction cloning reveals novel genes whose temporal and spatial expression indicates association with trophoblast invasion. Dev Biol 222(1):158–169. https://doi.org/10.1006/dbio.2000.9705
91. Yuan H, Wang X, Shi C, Jin L, Hu J, Zhang A, Li J, Vijayendra N, Doodala V, Weiss S, Tang Y, Weiner LM, Glazer RI (2018) Plac1 is a key regulator of the inflammatory response and immune tolerance in mammary tumorigenesis. Sci Rep 8(1):5717. https://doi.org/10.1038/s41598-018-24022-w
92. Sarker S, Scholz-Romero K, Perez A, Illanes SE, Mitchell MD, Rice GE, Salomon C (2014) Placenta-derived exosomes continuously increase in maternal circulation over the first trimester of pregnancy. J Transl Med 12:204. https://doi.org/10.1186/1479-5876-12-204
93. Saadeldin IM, Oh HJ, Lee BC (2015) Embryonic-maternal cross-talk via exosomes: potential implications. Stem Cells Cloning Adv Appl 8:103–107. https://doi.org/10.2147/SCCAA.S84991
94. Tong M, Chamley LW (2015) Placental extracellular vesicles and feto-maternal communication. Cold Spring Harbor Perspect Med 5(3):a023028. https://doi.org/10.1101/cshperspect.a023028
95. Lai A, Rice G, Salomon C (2017) Specific isolation of placenta-derived exosomes from maternal circulation. Placenta 57:300. https://doi.org/10.1016/j.placenta.2017.07.242
96. Austgulen R, Isaksen CV, Chedwick L, Romundstad P, Vatten L, Craven C (2004) Pre-eclampsia: associated with increased syncytial apoptosis when the infant is small-for-gestational-age. J Reprod Immunol 61(1):39–50. https://doi.org/10.1016/j.jri.2003.10.001
97. Sekizawa A, Farina A, Sugito Y, Matsuoka R, Iwasaki M, Saito H, Okai T (2004) Proteinuria and hypertension are independent factors affecting fetal DNA values: a retrospective analysis of affected and unaffected patients. Clin Chem 50(1):221–224. https://doi.org/10.1373/clinchem.2003.023259
98. Concu M, Banzola I, Farina A, Sekizawa A, Rizzo N, Marini M, Caramelli E, Carinci P (2005) Rapid clearance of mRNA for PLAC1 gene in maternal blood after delivery. Fetal Diagn Ther 20(1):27–30. https://doi.org/10.1159/000081365
99. Purwosunu Y, Sekizawa A, Farina A, Wibowo N, Okazaki S, Nakamura M, Samura O, Fujito N, Okai T (2007) Cell-free mRNA concentrations of CRH, PLAC1, and selectin-P are increased in the plasma of pregnant women with preeclampsia. Prenat Diagn 27(8):772–777. https://doi.org/10.1002/pd.1780
100. Avila L, Yuen RK, Diego-Alvarez D, Penaherrera MS, Jiang R, Robinson WP (2010) Evaluating DNA methylation and gene expression variability in the human term placenta. Placenta 31(12):1070–1077. https://doi.org/10.1016/j.placenta.2010.09.011
101. Fujito N, Samura O, Miharu N, Tanigawa M, Hyodo M, Kudo Y (2006) Increased plasma mRNAs of placenta-specific 1 (PLAC1) and glial cells-missing 1 (GCM1) in mothers with pre-eclampsia. Hiroshima J Med Sci 55(1):9–15
102. Kodama M, Miyoshi H, Fujito N, Samura O, Kudo Y (2011) Plasma mRNA concentrations of placenta-specific 1 (PLAC1) and pregnancy associated plasma protein A (PAPP-A) are higher in early-onset than late-onset pre-eclampsia. J Obstet Gynaecol Res 37(4):313–318. https://doi.org/10.1111/j.1447-0756.2010.01349.x
103. Ibanoglu MC, Ozgu-Erdinc AS, Uygur D (2018) Maternal placi protein levels in early- and late-onset preeclampsia. Ginekol Pol 89(3):147–152. https://doi.org/10.5603/GP.a2018.0025
104. Matteo M, Greco P, Levi Setti PE, Morenghi E, De Rosario F, Massenzio F, Albani E, Totaro P, Liso A (2013) Preliminary evidence for high anti-PLAC1 antibody levels in infertile patients with repeated unexplained implantation failure. Placenta 34(4):335–339. https://doi.org/10.1016/j.placenta.2013.01.006
105. Liso A, Massenzio F, Stracci F (2017) PLAC1 immunization does not induce infertility in mice. Immunotherapy. https://doi.org/10.2217/imt-2017-0019
106. Kotto-Kome AC, Silva C, Whiteman V, Kong X, Fant ME (2011) Circulating anti-PLAC1 antibodies during pregnancy and in women with reproductive failure: a preliminary analysis. ISRN Immunol 2011:5. https://doi.org/10.5402/2011/530491
107. Li Q, Liu M, Wu M, Zhou X, Wang S, Hu Y, Wang Y, He Y, Zeng X, Chen J (2018) PLAC1-specific TCR-engineered T cells mediate antigen-specific antitumor effects in breast cancer. Oncol Lett 15(4):5924–5932
108. Cardillo TM, Govindan SV, Sharkey RM, Trisal P, Goldenberg DM (2011) Humanized anti-Trop-2 IgG-SN-38 conjugate for effective treatment of diverse epithelial cancers: preclinical studies in human cancer xenograft models and monkeys. Clin Cancer Res 17(10):3157–3169
109. Bayat AA, Ghods R, Shabani M, Mahmoudi AR, Yeganeh O, Hassannia H, Sadeghitabar A, Balay-Goli L, Noutash-Haghighat F, Reza Sarrafzadeh A (2015) Production and characterization of monoclonal antibodies against human prostate specific antigen. Avicenna J Med Biotechnol 7(1):2
110. Cardillo TM, Govindan SV, Sharkey RM, Trisal P, Arrojo R, Chang C-H, Liu D, Rossi EA, Goldenberg DM (2015) Sacituzumab govitecan (IMMU-132), an anti-Trop-2/SN-38 antibody–drug conjugate: characterization and efficacy in pancreatic, gastric, and other cancers. Bioconjug Chem 26(5):919–931
111. Chari RVJ (2008) Targeted cancer therapy: conferring specificity to cytotoxic drugs. Acc Chem Res 41(1):98–107
112. Van der Meel R, Vehmeijer LJC, Kok RJ, Storm G, Van Gaal EVB (2013) Ligand-targeted particulate nanomedicines undergoing clinical evaluation: current status. Adv Drug Deliv Rev 65(10):1284–1298
113. Xiao Z, Jackson D, Tice DA (2013) EphA2 immunoconjugate. In: Phillips GL (ed) Antibody-drug conjugates and immunotoxins: from pre-clinical development to therapeutic applications, 1st edn. Springer, New York, pp 241–253