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PELP1 - A Key Mediator of Estrogen Signaling and Actions in the Brain

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Abstract

Proline-, Glutamic acid-, and Leucine-rich Protein 1 (PELP1) is an estrogen receptor (ER) coregulator protein identified by our collaborative group. Work from our lab and others has shown that PELP1 is a scaffold protein that interacts with ERs, kinase signaling factors, as well as proteins involved in chromatin remodeling and DNA repair. Its role in mediating 17 β -Estradiol (E2) signaling and actions has been heavily studied in cancer cells, but only recently has attention turned to its role in the brain. In this review, we discuss the tissue, cellular and subcellular localization of PELP1 in the brain. We also discuss recent evidence from PELP1 forebrain-specific knockout mice that demonstrate a critical role of PELP1 in mediating both extranuclear and nuclear ER signaling in the brain, as well as E2-induced neuroprotection, anti-inflammatory effects, and regulation of cognitive function. Finally, the PELP1 interactome and unique gene network regulated by PELP1 in the brain is discussed, especially as it provides new insights into PELP1 biology, protein interactions, and mechanisms of action in the brain. As a whole, the findings discussed in this review indicate that PELP1 functions as a critical ER coregulator in the brain to mediate E2 signaling and actions.

Keywords

PELP1; estrogen; coregulator; brain; signaling; neuroprotection

Introduction

The steroid hormone, 17 β -estradiol (E2) is primarily produced in the ovaries in females and circulates in the bloodstream to regulate various target tissues, including the brain [1]. As a

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lipophilic steroid hormone, E2 can enter cells and bind to estrogen receptors (ER) in extranuclear and nuclear compartments to exert its actions. While much is known on how E2 exerts estrogen receptor-mediated actions to regulate gene transcription, comparatively less is known about how E2 exerts rapid extranuclear signaling in cells.

Evidence of E2 Neuroprotective Effects

A large number of clinical and basic science studies have shown gender differences and implicated a neuroprotective role for E2 in neurodegenerative disorders such as Alzheimer's disease (AD) [2–5], Parkinson's disease [6] [1] and stroke [7–13]. E2 neuroprotection has been suggested to involve mediation by both the classical nuclear and extranuclear estrogen receptors, ESR1 (also known as ER α) and ESR2 (also known as ER β) [14–22]. In addition, emerging evidence suggests that G-protein coupled estrogen receptor-1 (GPER1, also known as GPR30) may also participate as an extranuclear receptor for estrogen to mediate the neuroprotective actions of E2 [23–25].

Multiple mechanisms of E2 neuroprotection

The mechanisms underlying E2 neuroprotection have been studied extensively in recent years. The findings from these studies suggest that E2 neuroprotection is mediated via regulatory effects upon multiple pathways and processes in cells [26], including: 1) genomic nuclear-receptor mediated signaling that regulates transcription of pro-survival, anti-apoptotic, and anti-inflammatory factors, 2) rapid extranuclear receptor-mediated signaling that enhances pro-survival kinase signaling pathways that can crosstalk to the nucleus and control transcription of protective and anti-inflammatory factors, 3) anti-oxidative stress effects that are mediated by suppression of free radical generation through regulatory effects on membrane NADPH oxidase- and mitochondrial-generating reactive oxygen species systems [17, 26–28], and 4) enhancement/preservation of mitochondrial bioenergetics [27], which could help cells survive stressful situations and be more resistant to damage.

It has been recently reported by our lab that the estrogen receptor co-regulator, Proline-, Glutamic acid-, and Leucine-rich Protein 1 (PELP1) has a critical role in mediating E2 signaling and actions in the brain. This mini-review will discuss the latest findings in the field that shed new light on the understanding of PELP1 structure, function and signaling in the brain, particularly as it relates to E2 signaling, and its neuroprotective, anti-inflammatory and cognitive-enhancing actions.

PELP1 – a novel ER coregulator

Investigations into the signaling mechanisms underlying E2 actions have revealed that E2-induced ER transcriptional activity can be influenced by “coregulator” proteins, which function as “coactivators” or “corepressors” [29]. Functionally, coregulator proteins act as a facilitator, modulator, or bridge to form large protein complexes and regulate target gene activity on chromatin. The ER-co-regulator protein association thus leads to formation of an “ER signalosome”, which facilitates both ER-nuclear and -extranuclear receptor-mediated action. To date, the majority of work on ER co-regulators has been performed in the cancer field, with little known on their function in the brain. However, recent work by our group led

to the cloning and characterization of a novel ER co-regulator that is highly expressed in the brain, called PELP1 [30–32], and which appears to play a key role in E2 signaling in the brain.

PELP1 Structure

Human PELP1 is localized on the chromosomal region 17p13.2 and encodes a 1130 amino acid protein [33]. The PELP1 gene is highly conserved across species. In humans, both a long (3.8 Kb) and a short (3.4 Kb) transcript have been identified. The long transcript is an immature transcript and has an extra 435bp intron. The short isoform is the mature transcript and is widely expressed in tissues throughout the body. PELP1 lacks known enzymatic activity, and its action in cells appears to be due to the many protein-protein interaction motifs in its structure, which allow it to function as a scaffolding protein coupling various proteins to transcription factors, nuclear receptors, kinases, and other proteins [33] (Figure 1). For instance, PELP1 contains 10 nuclear receptor (NR)-interacting boxes (LXXLL motifs) that allow its interactions with nuclear receptors, including estrogen receptors [30]. In addition, the C-terminus of PELP1 contains 70 acidic amino acids that functions as a histone-binding region (Figure 1) [34, 35]. PELP1-mediated ribosomal functions occur via its two nucleolar domains (Nuc 202) [36]. Furthermore, PELP1 contains several consensus PXXP motifs. It is at these motifs that it interacts with proteins containing Src homology 3 (SH3) domains such as kinases and other proteins. PELP1 also contains several conserved protein-protein interaction motifs that bind to forkhead-associated (FHA), Src homology 2 (SH2), PDZ, and WW domains. Collectively, these motifs allow PELP1 to interact with a large interactome, which will be discussed in detail in a subsequent section below.

PELP1 Expression

PELP1 expression is highest in the brain, testes, ovaries, and uterus [30, 31, 37, 38]. In the brain, PELP1 has been reported to be expressed in many regions, including the hippocampus, hypothalamus, cerebellum, cerebral cortex, amygdala, and bed nucleus of the stria terminalis [31, 32, 38, 39]. In the mammary gland, PELP1 expression is developmentally regulated [30]. Further work by our group has shown that PELP1 is an estrogen receptor (ESR) target gene, with two estrogen-response element (ERE) half sites in the promoter, and is upregulated by both ESR1 and ESR2 [40]. Intriguingly, PELP1 can exhibit both cytoplasmic and nuclear localization, indicating that it could mediate both extranuclear and nuclear actions of E2 [30]. In the nucleus, PELP1 has been demonstrated to localize in the chromatin, nucleoplasm, and nuclear matrix [35].

PELP1 Regulation

Post-translational phosphorylation is the main mechanism of regulation of PELP1 activity. Phosphorylation can control localization of PELP1, its interaction with adaptor proteins, and its stability [33] [41, 42]. PELP1 is phosphorylated by both hormonal and growth factor signals. Phosphorylation of PELP1 can be promoted by growth factors via protein kinase A (PKA) at Ser³⁵⁰, Ser⁴¹⁵, and Ser⁶¹³ (Figure 1) [43]. Recent work by our group demonstrated that glycogen synthase kinase 3 β (GSK3 β) phosphorylates PELP1 at Thr⁷⁴⁵ and Ser¹⁰⁵⁹ in the brain and thereby controls stability of PELP1 [42]. Phosphorylation of PELP1 at Ser⁴⁷⁷ and Ser⁹⁹¹ by cyclin dependent kinases (CDKs) has been reported to occur in a cell cycle-

dependent manner [44]. Finally, it has been reported that DNA damage induced kinases (ATM, ATR) can also phosphorylate PELP1 on Ser¹⁰³³ [45]. Functionally, phosphorylation of PELP1 can lead to coupling of physiological signals to nuclear receptors and transcriptional factors.

PELP1 Interactome

PELP1 can interact with many proteins in the cell. Several studies have shown that it interacts with and functions as a co-regulator of several nuclear receptors, such as ESR1 [46], ESR2 [47], estrogen-related receptor α (ERR α) [48] progesterone receptor (PR) [49], glucocorticoid receptor (GR) [50], androgen receptor (AR) [51], and retinoid X receptor (RXR) [52]. In addition to nuclear receptors, PELP1 can also serve as a coregulator of transcription factors, including activator protein 1 (AP1), specificity protein 1 (SP1), nuclear factor κ B (NF- κ B) [34], signal transducer and activator of transcription (STAT3) [53] and four and a half LIM domains 2 (FHL2) [51]. While the LXXLL motifs of PELP1 are known to interact with liganded steroid receptors, additional protein–protein interactions other than LXXLL motifs appear to mediate interaction with unliganded steroid receptors and other transcription factors [33].

Furthermore, PELP1 can interact with several key players of cell cycle progression, as well as other kinases and growth factors, including retinoblastoma protein (pRb) [54], cyclin-dependent kinase 2 (CDK2), and -4 (CDK4) [44], E2F1 and p53 [45, 55], c-Src [56], phosphoinositide 3-kinase (PI3K) [57], epidermal growth factor receptor (EGFR) [41], integrin-linked kinase (ILK1) [56], mechanistic target of rapamycin (mTOR) [58] and GSK3 β [42]. These PELP1 interactions suggest it has a role in mediating hormonal signaling cross talk with cell cycle machinery.

PELP1 is also known to have several epigenetic regulation functions as it interacts with chromatin-modifying complexes, including CBP/p300 [46], histone deacetylase 2 [34, 46], histones [34, 35], and Sumo-2, [59] among others. After formation of an interactome by PELP1 with one or more of the above-mentioned factors, it can exert several biological functions. These functions include ER-mediated genomic and non-genomic signaling, cell cycle regulation, chromatin modifications, histone modifications, DNA damage responses, RNA splicing, ribosomal biogenesis, and several neurological functions as well [33] (for detailed review).

PELP1 and cancer

Before discussing the neural roles of PELP1 in detail, the role of PELP1 in cancer will be reviewed briefly, as much of what we know about the biology of PELP1 has been derived from work in this area. From this work, it is now well accepted that PELP1 functions as a proto-oncogene, and provides cancer cells with advantages in both growth and survival [60–63]. Work from several laboratories has implicated a role for PELP1 in progression of multiple types of cancers including salivary, breast, lung, pancreas, colon, endometrial, ovarian, prostate, and brain [33] [64]. Extranuclear signaling of PELP1 plays a crucial role in metastasis [60]. Dysregulation of PELP1 signaling is responsible for development of hormonal therapy resistance [58]. Several phytoestrogens and resveratrol can lead to

recruitment of PELP1 to the autophagosome and thereby establish homeostasis in cancer cells [65, 66]. Besides its role in cancer metastasis proliferation and therapeutic targeting, PELP1 also plays a crucial role in breast cancer prognosis. PELP1 expression is an independent predictor of shorter breast cancer specific survival and disease free interval [67]. Another example of this is the correlation between cytoplasmic levels of PELP1 and poor response of patients to tamoxifen [68]. For further discussion of the role of PELP1 in cancer progression, treatment and prognosis, the reader is referred to previous reviews on the subject [33, 62, 69].

PELP1 – a key mediator of E2 signaling and actions in the brain

As mentioned previously, PELP1 is widely expressed in many regions of the brain of both rodents and monkeys, including key forebrain regions such as the hippocampus, hypothalamus and cerebral cortex [31, 32, 38, 39]. Subcellular localization studies revealed that PELP1 is highly localized in the cell nucleus of neurons, with a smaller amount located in the cytoplasm [32]. PELP1 has a nuclear localization signal that targets it to the nucleus [33]. In addition, hepatocyte growth factor-regulated tyrosine kinase substrate (HRS) interacts with PELP1 and can sequester it in the cytoplasm [6]. Electron microscopy studies further showed that PELP1 is localized in the cell membrane, dendritic shafts and pre- and post-synaptic terminals of neurons [31]. In addition to localization in neurons, PELP1 is also expressed in astrocytes [38]. Furthermore, PELP1 expression is reportedly markedly decreased in the brain of aged mice [70]. Co-localization studies have further revealed that PELP1 is co-localized in ESR1-positive cells in various brain regions [31]. Currently, it is not known whether PELP1 similarly co-localizes with ESR2 or GPER1 in the brain.

Establishment of PELP1 forebrain-specific knockout mouse model

In order to better elucidate the role of PELP1 in E2 genomic and non-genomic signaling and actions in the brain, our group recently created a PELP1 forebrain-specific knockout mouse model (PELP1 FBKO) using Cre/loxP technology [42]. The strategy involved deletion of exon 1 of PELP1 leading to a premature stop codon on the open reading frame. To target PELP1 deletion to the forebrain, PELP1 loxp/loxp mice were bred with Cre mice that expressed Cre under the control of the CaMKII α (Calcium/calmodulin-dependent protein kinase type II alpha chain) promoter, which is specifically expressed in excitatory neurons of the forebrain. The CaMKII α -Cre transgene is not expressed until after birth, thereby allowing normal embryonic development of the transgenic mice. Western blot analysis, PCR and immunohistochemistry confirmed that PELP1 knockout was specific for the forebrain and did not occur in other brain regions or in the periphery [42]. PELP1 FBKO mice were viable, with no obvious gross immunological, reproductive, or neurological abnormality or phenotype.

PELP1 mediates rapid extranuclear E2 signaling

To study the role of PELP1 in E2 effects upon rapid extranuclear signaling, gene expression, neuroprotection and cognition, we utilized a global cerebral ischemia (GCI) mouse model of brain ischemia, which causes extensive damage to the highly vulnerable hippocampus CA1 region. Using the GCI model, our studies revealed that E2-mediated extranuclear signaling

in the hippocampus to rapidly activate pro-survival ERK and Akt signaling was *lost* in PELP1 FBKO mice, as compared to FLOX control mice [42]. In addition, E2's ability to suppress activation of the pro-apoptotic factor, JNK was also lost in the PELP1 FBKO mouse. These results were also confirmed using an *acute* PELP1 antisense knockdown approach after GCI in a rat model [42]. Based on these results, it was suggested that E2 rapid signaling in the brain is likely due to the ability of PELP1 to form a scaffolding complex between ER and kinase signaling pathway proteins. Indeed, further studies showed that E2 enhanced PELP1 interaction with ESR1, Src and p85 subunit of PI3 kinase in the hippocampus rapidly after GCI [42]. As a whole, these findings suggest that PELP1 is essential for mediating E2 rapid, extranuclear activation of prosurvival ERK and Akt in the brain after an ischemic injury [42]. Furthermore, these results in the brain agree well with previous work demonstrating that PELP1 mediates E2-induced activation of MAPK and PI3K-Akt signaling in cancer cells [71].

PELP1 and GSK3 β -Wnt- β -catenin signaling

In addition to regulating E2 activation of prosurvival kinase signaling pathways, PELP1 also mediates E2 inhibition of neurodegenerative kinase signaling pathways in the brain such as GSK3 β . GSK3 β is a key mediator of cell death in several neurodegenerative conditions including cerebral ischemia [72, 73] and Alzheimer's disease (AD) [74]. Inhibition of GSK3 β has been shown to improve functional recovery after cerebral ischemia. Several kinases like, Akt, p70S6K, p90RSK, PKA and, MAPK can phosphorylate GSK3 β leading to its inactivation [75]. Furthermore, E2 has been shown to increase phosphorylation of GSK3 β leading to its inhibition [76]. GSK3 β is also a key player in the Wnt signaling pathway where it can negatively regulate pro-survival signaling by phosphorylation of β -catenin, leading to its degradation. Studies from our lab have also shown that E2 can up-regulate β -catenin after GCI [3]. Based on the strong link between E2 and GSK3 β /Wnt-catenin signaling, we therefore used the PELP1 FBKO mice to examine the role of PELP1 in mediating E2 regulation of GSK-3 β and β -catenin in the hippocampus after GCI [42]. The results revealed that the ability of E2 to inactivate GSK3 β and increase pro-survival β -catenin levels in the hippocampus after GCI was also lost in the PELP1 FBKO mice [42]. To identify PELP1 interacting proteins in the brain, we utilized PELP1 pulldown and mass spectrometry. The proteomic interactor screening identified many PELP1-interacting proteins in the brain, including most interestingly, GSK3 β [42]. Further studies determined that GSK3 β binds to the 400–600-aa region of PELP1, with a minor interaction also noted at the 1–400-aa region of PELP1 [42]. Mapping studies revealed that GSK3 β phosphorylates PELP1 at Thr⁷⁴⁵ and Ser¹⁰⁵⁹ (Figure 1), which leads to its degradation [42]. These results revealed that PELP1 is a novel substrate of GSK3 β , and indicated that an autocrine loop, involving E2-ER-GSK3 β signaling may be involved in modulating the levels of PELP1 at the site of neuronal damage.

PELP1 mediates E2 neuroprotection and cognition after ischemic injury

It is known that E2 can decrease neuronal damage and increase the number of surviving neurons after cerebral ischemia, as well as preserve cognitive function [1, 3, 8]. Therefore, we utilized the PELP1 FBKO mice to determine the importance of PELP1 in mediating these key functional outcomes after cerebral ischemia. The results revealed that E2 treatment

after cerebral ischemia improved neuronal survival and cognitive outcome in terms of spatial learning and memory. This ability of E2 to enhance neuronal survival and preserve cognitive function after GCI was lost in the PELP1 FBKO mice [42]. The role of PELP1 in mediating the neuroprotective and cognitive effects of E2 were also confirmed using an *acute* PELP1 antisense knockdown approach after GCI in a rat model. Taken as a whole, these results suggest that PELP1 mediates E2 rapid prosurvival signaling in the brain, as well as its neuroprotective and cognitive preserving actions after cerebral ischemia.

PELP1 regulates unique gene networks in the brain

To further enhance our understanding of the biology of PELP1 in the CNS, we performed RNA-sequencing analysis of hippocampal mRNA collected from E2-treated Floxed and PELP1 FBKO mice at 24 h after GCI [42]. RNA Seq analysis revealed that 229 genes (two-fold changes with $P < 0.01$) were differentially expressed in PELP1 FBKO mice as compared to the Floxed animals. Among these, 167 were upregulated and 62 genes were down-regulated in PELP1 FBKO. RT-qPCR analysis of a subset of the differentially regulated genes confirmed the RNA-Seq results. The majority of genes that were altered in PELP1 FBKO mice were involved in tissue development and function, cellular assembly and organization, neurological diseases and inflammation. These altered genes in PELP1 FBKO mice were part of key cellular pathways including neuroinflammation/neuronal death, chemokine signaling, Wnt pathway, cellular movement, cell-to-cell interactions, death, apoptosis, survival and DNA repair [42]. The identification of the inflammatory pathway as a major PELP1-regulated pathway in the brain was interesting. In follow-up studies, we confirmed that E2 strongly suppresses activation of a major pro-inflammatory pathway in the brain, the NLRP3 inflammasome pathway [77]. Further work using PELP1 FBKO mice confirmed that PELP1 is essential for E2 suppression of the NLRP3 inflammasome pathway, including downstream pro-inflammatory cytokine production. As a whole, these recent findings from our lab suggest an essential role for PELP1 in E2-mediated rapid extranuclear signaling, genomic signaling, neuroprotection, cognitive function and neuroinflammation in the ischemic brain [42, 77].

Conclusions

While much of what we know about PELP1 has come from studies in cancer cells, there is now growing evidence that PELP1 has a key role in mediating beneficial E2 signaling and actions in the brain. A significant advance in elucidating the roles and functions of PELP1 in the brain came from the generation of a novel PELP1 FBKO animal model. Use of the PELP1 FBKO mouse has revealed that PELP1 is critical for mediating E2 regulation of rapid extranuclear prosurvival signaling in the brain, as well as E2-induced neuroprotection and cognitive function in the hippocampus after ischemic injury. Since PELP1 lacks any known enzyme activity, its effects in the brain appear to be due to its ability to interact with many different proteins in the cell and function as a scaffold protein. RNA-Seq analysis further revealed that PELP1 regulates many genes in the brain, including those involved in neurological diseases and inflammation, Wnt signaling, and other key processes of the cell. While much has been learned on the role of PELP1 in the brain, considerable more remains to be elucidated. For instance, additional studies are needed to elucidate whether PELP1

exerts important epigenetic functions in the brain, since it binds to histones and interacts with many chromatin-remodeling enzymes. Furthermore, the role of PELP1 in mediating other nuclear receptor functions in the brain, in addition to estrogen receptors, also remains to be explored. Nevertheless, taken as a whole, the studies presented in this minireview suggest an emerging important role of PELP1 in mediating E2 signaling and actions in the brain.

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Abbreviations

E2	17 β -Estradiol
AD	Alzheimer's disease
CaMKIIα	Calcium/calmodulin-dependent protein kinase type II alpha chain
CDKs	Cyclin dependent kinases
ER	Estrogen receptor
ERK	Extracellular signal-regulated kinases
FBKO	Forebrain knockout
FHA	Forkhead-associated
GP1R1	G-protein coupled estrogen receptor-1
GCI	Global cerebral ischemia
GSK3β	Glycogen synthase kinase 3 β
JNK	c-Jun N-terminal kinases
MAPK	Mitogen activated protein-kinases
NLRP3	NOD-like receptor protein 3
PELP1	Proline-, Glutamic acid-, and Leucine-rich Protein 1
PI3K	Phosphatidylinositol 3-kinase
PKA	Protein kinase A
Akt	Protein Kinase B
SH2	Src homology 2
SH3	Src homology 3

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Figure 1. Schematic representation of PELP1 structure and posttranslational modification
 Proline-, glutamic acid-, and Leucine-rich protein 1 (PELP1) contains 10 LXXLL nuclear binding motifs, a nuclear localization signaling domain, a 70 amino acid long histone binding domain. Several known phosphorylation sites for maintenance of protein function and stability are also shown. (PKA – Protein kinase A, CDK – Cyclin dependent kinase, GSK3β – Glycogen synthase kinase 3 beta, ATM – DNA damage induced kinase).