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PELP1 – A Novel Estrogen Receptor-Interacting Protein

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Abstract

PELP1 (proline-, glutamic acid-, and leucine-rich protein-1) is a novel estrogen receptor (ER)-interacting protein that has been implicated to be important for mediation of both the genomic and nongenomic signaling of 17 β -estradiol (E2). PELP1 contains ten nuclear receptor-interacting boxes (LXXLL motifs), which allow it to interact with ER and other nuclear hormone receptors, a zinc finger, a glutamic acid-rich domain, and two proline-rich domains. The proline-rich regions contain several consensus PXXP motifs, which allow PELP1 to couple the ER with SH3 domain-containing kinase signaling proteins, such as Src and PI3K P85 regulatory subunit. PELP1 is expressed in many different brain regions, including the hippocampus, hypothalamus, and cerebral cortex. Further work has demonstrated that PELP1 is colocalized with ER- α in neurons in various brain regions. PELP1 is primarily expressed in neurons, with some expression also observed in glia. Subcellular localization studies revealed that PELP1 is highly localized in the cell nucleus of neurons, with some cytoplasm localization as well, and PELP1 is also localized at synaptic sites. Work in other tissues has demonstrated that PELP1 is critical for nongenomic and genomic signaling by E2, as PELP1 knockdown studies significantly attenuates E2-induced activation of ERK and Akt signaling pathways, and inhibits E2 genomic transcriptional effects on gene expression in breast cancer cells. Preliminary studies in the brain, suggests that similar roles may exist for PELP1 in the brain, but this remains to be established, and further work to characterize the precise roles and functions of PELP1 in the brain are needed.

Keywords

Estradiol; nongenomic; genomic; signaling; hippocampus

INTRODUCTION

The steroid hormone, 17 β -estradiol (E2) is well known to exert regulatory effects in a variety of tissues in the body, including the brain, breast, cardiovascular system, uterus and bone. In the brain, E2 has been implicated to regulate such key processes as neuroprotection, synaptic

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plasticity, memory, reproduction and sexual behavior (Alkayed et al., 1998; Gore, 2001; Hewitt and Korach, 2003; Kelly et al., 2005; Brann et al., 2007). E2 has generally been thought to exert its actions through the classical genomic mechanism of direct control of gene expression via interaction with estrogen receptor (ER)- α and/or ER- β (O'Lone et al., 2004; Zhao et al., 2008). While it is clear that ERs function as ligand-dependent transcription factors, functional interaction of ERs with coregulators is required for optimal activation of E2 responsive genes (Smith and O'Malley, 2004). Along these lines, several studies have shown that ER functions are dependent on the formation of large, multi-component complexes in the nucleus (McKenna et al., 1999). ERs also participate in cytoplasmic and membrane-mediated signaling events (nongenomic signaling) by forming complexes with Src kinase, mitogen-activated protein kinase (MAPK), and phosphatidylinositol-3-kinase (PI3K) (Losel and Wehling, 2003; Bjornstrom and Sjoberg, 2005; Song et al., 2005; Cheskis et al., 2008). A major area of research has been to identify ER-interacting proteins that form scaffold/transcriptional regulatory complexes with ER and thus modulate the genomic and nongenomic signaling by E2 in cells. This review will focus on a specific ER-interacting protein called PELP1 (proline-, glutamic acid-, and leucine-rich protein-1), which has been implicated to mediate both the genomic and nongenomic signaling effects of E2. Much of what we know about this novel ER-interacting protein has come from studies in breast cancer cells, where it has been implicated to play a role in ER-positive breast cancers. However, PELP1 is expressed in the brain and other tissues in the body, and thus likely has a role in mediating *physiological* effects of E2 in the body. In subsequent sections, we will review current knowledge of the structure/function of PELP1, identification of PELP-1 interacting proteins, mechanisms of post-transcriptional modifications of PELP1, as well as PELP1 expression, localization and potential functions in the brain.

PELP1 Cloning and Protein Domain Structure

Cloning

PELP1 was first identified as a 160 kDa interacting protein in GST-SH2 pull-down assays using as bait the SH2 domain of Lck tyrosine protein kinase (Joung et al., 1996). Vadlamudi et al. (2001) subsequently cloned the human PELP1 cDNA by using peptide sequences derived from the purified 160-kDa protein as oligonucleotide probes. Initial studies using the PELP1 clone (3.8 kb) resulted in generation of a 160-kDa protein product and identification of coactivator activity for ER- α transactivation (Vadlamudi et al., 2001). Work by another group identified a protein identical to PELP1 at the amino acid level, but differed slightly in cDNA length (3.4 kb), which the researchers presumed was a new protein and named it modulator of nongenomic actions of estrogen receptor (MNAR) (Wong et al., 2002). However, both the PELP1 and MNAR gene sequences map to the same chromosomal region and have identical sequences except for an additional 435-bp region in PELP1, which later work showed was due to isolation of an immature transcript for PELP1 that contained an extra 435-bp intron with consensus splice sites that artificially produced the disparity in length between the PELP1 and MNAR cDNAs (Balasenthil and Vadlamudi, 2003). Further studies confirmed that PELP1 and MNAR code for the same proteins, and subsequently the HUGO Gene Nomenclature Committee (HGNC) approved PELP1 as the approved symbol for PELP1/MNAR.

PELP1 Protein Domain Structure

As illustrated in Figure 1, examination of the protein domain structure of PELP1 revealed that PELP1 contains ten nuclear receptor-interacting boxes (LXXLL motifs), a zinc finger, a glutamic acid-rich domain, and two proline-rich domains (Figure 1 (Vadlamudi et al., 2001; Wong et al., 2002). The C-terminus contains a 70 amino acid sequence that functions as a histone-binding region (Choi et al., 2004; Nair et al., 2004). Of significant interest, the proline-rich regions contained several consensus PXXP motifs, which suggest capability to interact

with signaling proteins containing SH3 domains. Analysis of the primary PELP1 sequence revealed that PELP1 contains several conserved protein-protein interaction motifs that bind to Forkhead associated (FHA) domains, src-homology-2 (SH2) and src-homology-3 (SH3) domains, PSD95-Dlg-Zo1 (PDZ) domains, and WW domains (domains that interact with short proline-rich sequences). Human PELP1 encodes a protein of 1130 amino acids and migrates on sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels as a 160-kDa protein. Subsequent work by our laboratories led to the cloning of rat and monkey PELP1, which display 86% and 98% homology to the human PELP1 gene, respectively, and which were shown to contain the same protein domain motifs as human PELP1, e.g. glutamic acid clusters, LXXLL domain motifs, and PXXP domain motifs (Khan et al., 2005; Khan et al., 2006).

PELP1 Interacting Proteins

PELP1 has been shown to interact with both ER- α and ER- β via its nuclear receptor-interacting boxes (LXXLL). Specifically, LXXLL motifs 4 and 5 have been shown to be important for mediating binding of PELP1 to ER- α (Barletta et al., 2004). Furthermore, Vadlamudi et al (2001) demonstrated that estrogen promotes PELP1 interaction with the AF2 domain of ER- α (Vadlamudi et al., 2001). PELP1 also interacts with ER- β (Wong et al., 2002). Using receptor subtype-specific ligands, it has been demonstrated that PELP1 acts as a coactivator for both ER- α and ER- β (Vadlamudi et al., 2004). Treatment of MCF-7 breast cancer or endometrial cancer cells with either PELP1 antisense oligonucleotides or PELP1 siRNA has been demonstrated to attenuate estrogen-induced gene expression of target genes (Vadlamudi et al., 2001; Wong et al., 2002). PELP1 also interacts with several other NRs, including androgen receptors, glucocorticoid receptors, and progesterone receptors, in a ligand-dependent manner (Vadlamudi et al., 2001; Wong et al., 2002; Nair et al., 2007). PELP1 has also been shown to associate with histones and can recruit other ER coregulators with histone acetyltransferase activity such as CBP and p300 (Nair et al., 2004). PELP1 also interacts with components of histone deacetylase complexes such as MTA1 and histone deacetylase 2 (Mishra et al., 2003; Choi et al., 2004). These findings suggest that PELP1 has a role in modulating local chromatin structure in the vicinity of nuclear receptor promoters (Nair et al., 2004). PELP1 also exists in a complex with methyltransferases and methylases, suggesting that PELP1 has a potentially important function in these complexes (Rosendorff et al., 2006).

Further work has shown that PELP1, via its PXXP motifs, interacts with cell signaling proteins such as Src and PI3K P85 regulatory subunit, which is consistent with a role of PELP1 in mediating estrogen-induced ERK and Akt activation in cells (Vadlamudi et al., 2001; Wong et al., 2002). In support of this, studies in breast cancer cells have shown that PELP1 modulates ER interaction with Src, promotes Src activity, and enhances ERK activation (Wong et al., 2002). PELP1 has also been shown to enhance PI3K activity and Akt activation in breast cancer cells (Vadlamudi et al., 2005a; Greger et al., 2007). Preliminary work from our laboratory show a similar formation of PELP1-ER-Src complex and PELP1-ER-PI3K-p85 complex in the hippocampus CA1 within 30 minutes following global cerebral ischemia, and that E2 enhances the complex formation, which correlates with estrogen-induced ERK and Akt activation following stroke (Figure 2). Further work is underway to determine whether PELP1 mediates estrogen-induced ERK and Akt activation in the brain in physiological as well as pathological situations.

PELP1 Expression and Localization

PELP1 contains a central consensus nuclear localization site and exhibits both nuclear and cytoplasmic localization in a variety of cells in the body (Vadlamudi et al., 2001; Nair et al., 2004; Khan et al., 2005; Khan et al., 2006). In hormonally responsive tissues, PELP1 has been shown to predominantly reside in the nucleus, but localization in the cytoplasm is also

observed, albeit to a lesser degree than nuclear localization (Vadlamudi et al., 2001; Khan et al., 2005; Vadlamudi et al., 2005a; Khan et al., 2006). A similar nuclear and cytoplasm localization of PELP1 has been demonstrated in neurons by electron microscopy (Figure 3) (Khan et al., 2006). Localization of PELP1 in dendritic shafts and at synaptic sites has also been described (Figure 3) (Khan et al., 2006), which is consistent with synaptic and dendritic localization of ER- α (Milner et al., 2001). The nuclear localization of PELP1 is consistent with its purported role in mediating genomic signaling by estrogen, while its cytoplasmic localization suggests a potential role in mediating fast, nongenomic signaling by estrogen. To further study the role of PELP1 localized in the cytoplasm, Vadlamudi et. al. (Vadlamudi et al., 2005a) created a mutant protein that was expressed only in the cytoplasm (PELP1-cyto) and then generated a model system wherein MCF-7 breast cancer cells were engineered to specifically express the mutant. PELP1-cyto cells exhibited increased association of PELP1 with Src, enhanced mitogen-activated protein kinase (MAPK) activation, and constitutive activation of Akt. The altered localization of PELP1 was sufficient to trigger the interaction of PELP1 with the p85 subunit of phosphatidylinositol-3-kinase (PI3K), leading to PI3K activation. In addition, PELP1 interacted with epidermal growth factor receptors and participated in growth factor-mediated ER transactivation functions. The mechanisms of how PELP1 is targeted to the cytoplasm is unclear, but recent work has shown that hepatocyte growth factor-regulated tyrosine kinase substrate (HRS), which is an early endosomal protein and PELP1-interacting protein, can sequester PELP1 in the cytoplasm (Rayala et al., 2006).

With respect to localization in the CNS, PELP1 has been shown to be expressed in many brain regions in both rodents and monkeys that are targets for E2 action, including the hippocampus, hypothalamus, cerebral cortex, cerebellum and pituitary (Khan et al., 2005; Khan et al., 2006). Double immunohistochemistry studies have revealed that PELP1 is colocalized in ER- α -positive cells in various brain regions, including a number of hypothalamic nuclei, cerebral cortex, hippocampus, amygdala, and bed nucleus of the stria terminalis. In contrast, few gonadotropin hormone releasing hormone (GnRH) neurons were observed to colocalize PELP1 in the preoptic area of the hypothalamus (Khan et al., 2005). It is thus possible that PELP1 helps mediate feedback control of GnRH secretion by E2 that is exerted via an *indirect* mechanism.

The colocalization of PELP1 with ER- β in the brain has not been assessed yet due to technical issues in finding suitable ER- β antibodies for double immunohistochemical colocalization studies. However, colocalization would be expected based on studies showing that PELP1 also forms a complex with ER- β , in addition to complex formation with ER- α (Vadlamudi et al., 2001; Wong et al., 2002). The colocalization of PELP1 with ER- α in the brain suggests that PELP1 may have an important role in mediating ER- α -mediated genomic and nongenomic signaling actions of estrogen in the brain. Thus, PELP1 may have a key role in estrogen actions in the brain to control synaptic plasticity, exert neuroprotection and modulate reproductive function. Further work is underway in our laboratories to address this key issue. Finally, work by our laboratory has also shown that PELP1 colocalizes with glucocorticoid receptors in monkey and mouse brain, suggesting an additional potential role for PELP1 in modulating glucocorticoid action (Khan et al., 2006).

Posttranslational Modification of PELP1

Recent work by several groups has demonstrated that PELP1 is a phosphoprotein and is phosphorylated on both serines/threonines and tyrosines. Analysis of PELP1 structure using protein phosphorylation site prediction software indicated that PELP1 contains several potential sites for phosphorylation, including 8 tyrosine kinase/phosphatase sites (recognized by EGFR, platelet-derived growth factor receptor, insulin receptor, Src, Jak2, and SHP1) and 207 serine/threonine kinase/phosphatase motifs (recognized by AKT, glycogen synthase

kinase, CDK, casein kinase 1, casein kinase 2, LKB1, mitogen-activated protein kinase [MAPK], protein kinase C, protein kinase A [PKA], and proline-directed kinases). A recent study by our group using MCF-7 breast cancer cells showed that PELP1 is phosphorylated by PKA at Ser-350, Ser-415, and Ser-613 (Nagpal et al., 2008). Furthermore, Ballif et. al. used phosphoproteomic analysis, which revealed that PELP1 is phosphorylated at Thr-745 in the developing brain (Ballif et al., 2004). Greger et. al. showed that interaction of PELP1 with ER- α , Src and PI3K-p85 subunit in MCF-7 cells and E2 activation of Akt required phosphorylation of PELP1 at Tyr920 (Greger et al., 2007). Additionally, hormones and growth factor appear to regulate PELP1 phosphorylation, as treatment with EGF promotes tyrosine as well as serine phosphorylation of PELP1 (Vadlamudi et al., 2005b). Estrogen treatment has also been shown to promote tyrosine phosphorylation of PELP1 at Tyr920 in MCF-7 breast cancer cells (Greger et al., 2007). Finally, preliminary work by our lab suggests that estrogen enhances both serine and tyrosine phosphorylation of PELP1 in the hippocampus CA1 following global cerebral ischemia, a finding that may have implications to the neuroprotective effects of estrogen in the brain [Zhang QG, Vadlamudi RK, and Brann DW unpublished observation].

PELP1 Target Genes

While PELP1 does not appear to have a DNA-binding domain, it does interact with ERs, suggesting that it enhances the transcription of estrogen target genes (Vadlamudi et al., 2001; Wong et al., 2002). In support of this contention, chromatin immunoprecipitation analysis has shown that PELP1 is recruited to the promoters of ER- α target genes, including progesterone receptor, pS2 and insulin-like growth factor (Nair et al., 2007). PELP1 also regulates cyclin D1 expression at the transcriptional level; such regulation may involve functional interactions between PELP1 and retinoblastoma protein (pRb) (Balasenthil and Vadlamudi, 2003). The interaction of PELP1 with pRb and regulation of cyclin D1 suggests that PELP1 may have a role in mediating estrogen regulation of cell cycle in cells. In support of this possibility, PELP1 has been shown to positively contribute to E2-mediated G1/S-phase progression and plays a permissive role in E2-mediated cell cycle progression in MCF-7 cells, presumably via its regulatory interactions with the pRb pathway (Balasenthil and Vadlamudi, 2003).

Estrogen stimulation leads to enhanced recruitment of PELP1 to the MTA3 promoter chromatin, implying a role for PELP1 in the regulation of metastasis associated 1 family, member 3 (MTA3) (Mishra et al., 2004). Additionally, growth factor signals promote PELP1 interactions with STAT3; these interactions play an important role in growth factor-mediated activation of STAT3 target genes, including cyclin D1, fos, and jun (Manavathi et al., 2005). Additionally, PELP1 overexpression in MCF-7 cells enhances phosphorylation of STAT3 via a Src-MAPK-dependent manner, and down-regulation of PELP1 interfered with recruitment of STAT3 to its target gene promoters (Manavathi et al., 2005). This may have application to the brain as well, as estrogen was recently demonstrated to enhance phosphorylation of STAT3 in the cerebral cortex following focal cerebral ischemia, an effect shown to be critical for the neuroprotective effects of estrogen (Dziennis et al., 2007). Studies to determine the role of PELP1 in estrogen-induced activation of STAT3 in the brain are needed, as are studies to determine the role of PELP1 in estrogen-induced transcription of genes in the brain.

Conclusions and Future Directions

PELP1 is a unique ER-interacting protein implicated to mediate *both* the genomic and nongenomic signaling actions of estrogen in cells. The role of PELP1 has been primarily explored in pathological conditions, such as in endocrine-regulated cancers, but it is widely expressed in the brain, colocalizes with ER- α , and forms a complex with ER- α , Src and PI3K-p85, suggesting it may mediate rapid signaling by estrogen in the brain. PELP1 also acts as a cofactor in the nucleus and evidence suggests that it is critical for the transcriptional effects of

estrogen. Future studies in the field are needed to focus on elucidating the role of PELP1 in genomic and nongenomic signaling by estrogen in the brain and on determining its importance in mediating estrogen neural functions such as modulation of synaptic plasticity, learning and memory, neuroprotection and reproductive function. Since PELP1 can also interact with other nuclear receptors such as androgen, progesterone and glucocorticoid receptors, future studies are also needed to address its importance in mediating the signaling and functions of these nuclear receptors. To help facilitate our understanding of the *physiological* roles of PELP1 in the brain and other tissues, our laboratories are collaborating to develop and characterize PELP1 conditional knockout animals. It is hoped that through such studies we will gain a new understanding of the physiological roles of PELP1 in the brain and body, and help elucidate further the signaling mechanisms whereby estrogen exerts its many regulatory effects throughout the body.

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Figure 1. Schematic diagram of PELP1/MNAR interacting proteins and functional domains
 PXXP, SH3, Src homology3 binding domain; LXXLL, nuclear receptor interacting domain; glu rich, histone binding domain; NLS, nuclear localization signal. Putative PELP1 phosphorylation sites (Ser, serine; Thr, threonine; Tyr, tyrosine) are indicated. Known PELP1 interacting proteins are shown and are grouped based on putative non genomic and genomic functions.

Figure 2. Preliminary co-immunoprecipitation analysis of PELP1-ER-Src and PELP1-ERPI3K-p85 complex in rat hippocampal CA1 from placebo- and 17 β -estradiol (E2)-treated ovariectomized rats following 4-vessel global cerebral ischemia

Sample proteins from placebo and E2-treated ovariectomized female rats were collected 30 min after cerebral ischemia, and were separately immunoprecipitated (IP) using antibody against ER α , PELP1 and Src, and then Western blot (WB) performed with indicated antibodies. At the time of ovariectomy, placebo or 17 β -estradiol (E2) time-release pellets (0.025mg; 21 day release pellet) were implanted subcutaneously in the upper mid-back region under the skin. The E2 pellets produce diestrus 1 level serum E2 levels (~10 pg/ml), which strongly protects the hippocampus CA1 from cerebral ischemia-induced neuronal cell death. Note that E2 strongly up-regulates PELP1-ER-Src and PELP1-ER-PI3K-p85 complex formation as compared to placebo control.

Figure 3. Subcellular localization of PELP1 in the rat brain

A Low-magnification view of silver-enhanced nanogold labeling of the PELP1 localization in the CA1 pyramidal cell layer of the hippocampus. **B** Electron micrograph of an individual pyramidal cell from the CA1 area showing nuclear and cytoplasmic compartments as well as various cytoplasmic organelles. **C**, **D** High-resolution electron micrographs showing PELP1-positive gold particles localized in nucleus and cytoplasm, respectively. **E–H** High-resolution electron micrographs showing localization of PELP1-positive gold particles in the dendritic shafts and pre- and postsynaptic terminals. Ds = Dendritic shafts; PSDs = postsynaptic density complexes; PSD = presynaptic dendritic vesicles; Ps = presynaptic vesicles. **I** Localization of PELP1 in an astrocyte. Scale bars represent 50 nm in **A** and 200 nm in **B–I**. With permission from Khan et. al. 2006.