Frontiers: PED/PEA-15, a multifunctional protein controlling cell survival and glucose metabolism

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PED/PEA-15 is a 15-kDa cytosolic protein featuring an NH2-terminal death effector domain (DED) and a COOH-terminal tail with irregular structure (47). PED/PEA-15 gene (Fig. 1A) maps on human chromosome 1q21-22, is highly conserved among mammals, and is ubiquitously expressed in human tissues (11). PED/PEA-15 gene is expressed to high levels in the nervous system (11). Indeed, PED/PEA-15 is a 15-kDa ubiquitously expressed protein implicated in a number of fundamental cellular functions, including apoptosis, proliferation, and glucose metabolism. PED/PEA-15 lacks enzymatic function and serves mainly as a molecular adaptor. PED/PEA-15 is an endogenous substrate for protein kinase C (PKC), calcium/calmodulin-dependent protein kinase II (CaMKII), and Akt. In particular, PKC phosphorylates PED/PEA-15 at Ser104 and CAM kinase II or Akt at Ser116, modifying its stability. Evidence obtained over the past 10 years has indicated that PED/PEA-15 regulates cell survival by interfering with both intrinsic and extrinsic apoptotic pathways. In addition, it may also control cell proliferation by interfering with ERK1/2-mediated pathways. Indeed, PED/PEA-15 has been identified as an ERK1/2 interactor, which modifies its subcellular localization and targeting to a specific subset of substrates. Increased PED/PEA-15 levels may affect tumorigenesis and cancer progression as well as sensitivity to anticancer agents. Moreover, PED/PEA-15 affects astrocyte motility and increases susceptibility to skin carcinogenesis in vivo. PED/PEA-15 expression is regulated at the transcriptional and the posttranslational levels. Increased PED/PEA-15 expression has been identified in individuals with type 2 diabetes early during the natural history of the disease. Evidence generated over the past 10 years indicated that this defect contributes to altering glucose tolerance by impairing insulin action and insulin secretion and might play a role in the development of diabetes-associated neurological disorders. Strategies are being devised to target key signaling events in PED/PEA-15 action aimed at improving glucose tolerance and at facilitating cancer cell death.

type 2 diabetes

PED/PEA-15 is a 15-kDa cytosolic protein featuring an NH2-terminal death effector domain (DED) and a COOH-terminal tail with irregular structure (47). PED/PEA-15 gene (Fig. 1A) maps on human chromosome 1q21-22, is highly conserved among mammals, and is ubiquitously expressed in human tissues (11). PED/PEA-15 gene is expressed to high levels in the nervous system (11). Indeed, PED/PEA-15 protein has initially been identified in [32P]phosphate-labeled astrocytes (1). These authors demonstrated PED/PEA-15 phosphorylation to occur in response to 12-O-tetradecanoylphorbol-13-acetate (TPA) exposure and named it phosphoprotein enriched in diabetes (9). More recent work has highlighted that PED/PEA-15 can block Ras suppression of the extracellular signal-regulated kinase (ERK) pathway (4, 17). Fine tuning of this pathway is key to a number of processes connected with eukaryotic cell growth (31), including gene transcription (40). Early experiments by Ramos and colleagues (45, 46) led us to recognize that PED/PEA-15 can block Ras suppression of integrin activation without blocking ERK activity. Subsequent studies in astrocytes revealed that PED/PEA-15 inhibits the translocation of ERK into the nuclei and cell proliferation. Indeed, PED/PEA-15 binds both ERK1 and ERK2 in yeasts independently of ERK phosphorylation (17). Nevertheless, PED/PEA-15 biphasohorylation negatively controls ERK binding (49), suggesting that growth factor activation of PKC, CaMKII, and PKB may facilitate the release of ERK. PED/PEA-15 features a leucine-rich nuclear export sequence (NES), which keeps it predominantly cytoplasmic despite its small size. Point mutation at a conserved site in the NES results in PED/PEA-15 nuclear accumulation (17). Importantly, this mutation disables PED/PEA-15’s inhibitory effect on ERK nuclear translocation, demonstrating that binding to the cytosolic protein PED/PEA-15 prevents nuclear translocation and functions of ERKs. Two PED/PEA-15 regions were found to be necessary for ERK2 binding (24). The E1 region encompasses several residues localized at the NH2-terminal DED of PED/PEA-15. The second domain, termed E2, localizes at the COOH terminus. Both of these domains have initially been identified by nuclear magnetic resonance footprinting and then functionally characterized by site-directed mutagenesis. In ad-
expression at high levels in astrocytes. These cells feature reduced sensitivity to Fas-mediated apoptosis. However, PED/PEA-15 does not affect the phosphorylation of RSK2, and the increase of its activity. PED/PEA-15 serves a scaffold that sequesters both ERK and RSK2 with no interaction with its DED.

Expression of PED/PEA-15 inhibits phosphorylation of nuclear, although not cytoplasmic, substrates of ERKs and inhibits its ERK-dependent transcription. The p90 ribosomal S6 kinase isozyme (RSK2) is a transcriptional regulator shown to serve as a substrate of ERK. RSK2, but not RSK1, shows cytoplasmic colocalization with PED/PEA-15. PED/PEA-15 was shown to coprecipitate with PED/PEA-15 and directly binds PED/PEA-15 COOH terminus with no interaction with its DED. Similarly to ERK, PED/PEA-15 blocks RSK2 nuclear accumulation following EGF stimulus, thereby impairing RSK2-dependent cAMP response element-binding protein transcription and phosphorylation of histone H3 (60). Further studies by Vaidyanathan et al. (59) showed that, in different cell types, PED/PEA-15 serves a scaffold that sequesters both ERK and RSK2 in the cytosol, inducing ERK binding to RSK2, the phosphorylation of RSK2, and the increase of its activity. However, PED/PEA-15 does not affect the phosphorylation of other ERK substrates, providing a model to understand how scaffold proteins can integrate signal transduction and generate specificity in the multipotent ERK signaling pathway.

PED/PEA-15 Control of Apoptotic Programs

Neurons feature exquisite sensitivity to apoptotic stimuli and easily start apoptotic programs in a number of pathological conditions. At variance, astrocytes are quite resistant to cell damage. Typically, these cells initiate reparative responses and reactive gliosis in response to nervous tissue damage (47). Reactive astrocytes feature upregulation of both tumor necrosis factor (TNF)α and apoptosis-signaling receptors of the TNF receptor superfamily, including TNR receptor 1 and Fas. However, these cells feature reduced sensitivity to Fas-mediated cytotoxicity. PED/PEA-15 is expressed at high levels in astrocytes. Its NH₂-terminal DED enables it to interact with different DED-containing signaling proteins, including Fas-associated protein with death domain (FADD) and FADD-like IL-1β-converting enzyme (FLICE), leading to the hypothesis that PED/PEA-15 may protect astrocytes from apoptotic insults (26). Indeed, astrocytes from PED/PEA-15-null mice were found to feature increased susceptibility to TNF-induced apoptosis (27). Subsequent studies revealed that PED/PEA-15 modulates Fas and TNFα-R-initiated signaling. Exposure to their ligands leads these receptors to interact with adaptor molecules, recruiting them to the plasma membrane (5). One such molecule is FADD. Upon interacting with Fas, FADD recruits further apoptosis-signaling molecules at the plasma membrane, producing a death-inducing signaling complex (DISC), where the initial activation of the caspase cascade occurs (38). In different cell types in addition to astrocytes, PED/PEA-15 was reported to inhibit apoptotic responses to Fas and TNFα. It has been shown that, in these cells, PED/PEA-15 stably associates with both FADD and the upstream caspase FLICE through the DED present in all of these proteins. In turn, this association interferes with formation of the DISC and displaces FADD-FLICE interaction and the induction of FLICE protease activity required to signal activation of downstream caspases and to elicit apoptotic responses (Fig. 2) (8).

PED/PEA-15 action on DISC function has been further addressed in studies aimed at clarifying astrocyte resistance to TNR-related apoptosis-inducing ligand (TRAIL). These studies revealed that TRAIL death receptors DR4 and DR5 are expressed weakly at the cell surface level and thus fail to mediate the assembly of the DISC in human astrocytes. DR5 overexpression restores TRAIL signaling pathways and sensitizes the human astrocytes to TRAIL-induced apoptosis, provided that PED/PEA-15 is inhibited (50). In other cell types, such as the human malignant glioma cells, sensitivity and resistance to TRAIL-induced apoptosis are, respectively, accompanied by low and high levels of PED/PEA-15 expression (13). Interestingly, genetic silencing of PED/PEA-15 by antisense oligonucleotides in TRAIL-resistant cells has been
shown to render them sensitive (22). Indeed, subsequent studies by Xiao et al. (68) demonstrated that, in the TRAIL-resistant glioma cells, PED/PEA-15 is abundantly represented at the DISC level.

Only the bisphosphorylated PED/PEA-15 was found to be present in the DISC, suggesting that phosphorylation is necessary for PED/PEA-15 antiapoptotic action. Indeed, PED/PEA-15 antiapoptotic action is dependent on PKC and no longer detectable upon exposure to PKC-inhibiting agents (8). In addition to causing increased PED/PEA-15 degradation, mutation at the Ser116 Akt/PKB phosphorylation site also weakens PED/PEA-15 antiapoptotic function, as measured in 293 cells upon serum deprivation. Consistently, in the U373MG glioma cells, Akt/PKB inhibition reduces PED/PEA-15 levels and increases TRAIL-induced apoptosis (56). The relevance of PED/PEA-15 phosphorylation for its function is confirmed by the findings of Estellés et al. (14). These authors showed that, in NIH-3T3 cells, PED/PEA-15 inhibits Fas- but increases TNF receptor 1-mediated caspase-8 activation. Interestingly, PED/PEA-15 phosphorylation is critical for its function in the Fas pathway but not for its death-inducing activity via TNF receptor 1 (14). These apparent discrepancies might result from the involvement of PED/PEA-15 in different molecular complexes regulating apoptosis and survival, which include interacting partners such as FADD, FLICE, ERK, and RSK.

Thus, PED/PEA-15 modulation of the extrinsic apoptotic pathway is tightly regulated. At least in part, this regulation depends on the cellular control of PED/PEA-15 Ser phosphorylation state by PKC and Akt/PKB.

Subsequent studies revealed that PED/PEA-15 may further restrain activation of the intrinsic apoptotic pathway. PED/PEA-15 inhibits stress-induced apoptotic responses following exposure to oxidative agents, serum deprivation, and anisomycin treatment (Fig. 2) (7). In addition, PED/PEA-15 blunts apoptotic bursts triggered by the release of proapoptotic mitochondrial proteins. One such protein has recently been identified in a yeast two-hybrid system as the PED/PEA-15 interactor Omi/HtrA2 (55). Omi/HtrA2 is a proapoptotic mitochondrial serine protease released in response to different cellular stresses. Omi/HtrA2 was thought to exert its proapoptotic function by both antagonizing the inhibitor of apoptosis protein and exerting its protease activity (52). It was later clarified that PED/PEA-15 serves as an Omi/HtrA2 substrate. Stress-induced release of Omi/HtrA2 in the cytoplasm results in binding and degradation of PED/PEA-15, thereby reducing its antiapoptotic action and triggering apoptotic programs (55).

Even more recent studies have documented that PED/PEA-15 regulates the ability of Bcl-2 to suppress Fas-induced apoptosis, indicating that it further serves as a switch determining whether, upon Fas exposure, cell apoptosis is signaled through a mitochondrionally independent type I or a mitochondrially dependent type II pathway (39). Thus, PED/PEA-15 has emerged as a major regulator of apoptosis, exerting its inhibitory action on both the extrinsic and the intrinsic pathways and switching apoptotic signal transduction from one pathway to another.

PED/PEA-15 in Neoplastic Transformation and Cancer Development

Mounting evidence indicates that the acquired ability to resist apoptosis is a hallmark of most, and perhaps all, types of...
cancer. As the understanding of how apoptosis is thwarted in cancer proceeds, it has also become more clear why many tumors are resistant to the cell suicide-inducing effects of radiation and chemotherapy. Therefore, not surprisingly, understanding the role of PED/PEA-15 in apoptosis has been paralleled by investigation on its potential significance in cancer development and treatment.

There is evidence in the literature suggesting that, particularly in tumors driven by oncogenic Ras, PED/PEA-15 acts as an ERK1/2 nuclear export factor and exerts tumor suppressor activity (19). At least under certain conditions, PED/PEA-15-induced cytosolic localization of phospho-ERKs might prevent oncogenic transformation and lead to cellular senescence. However, many published reports provide evidence that high levels of PED/PEA-15 expression associate with development of malignancy. Initial studies revealed that ped/pea-15 gene is overexpressed in human gliomas (22, 68) and in mammary carcinomas (25, 53, 57) and may mediate chemoresistance in these tumors (53). Results from these investigations also suggest that ped/pea-15 overexpression plays a causal role in development of gliomas and mammary carcinomas and may have a role in development of other human tumors as well. This possibility is further corroborated by expression profile analyses revealing ped/pea-15 overexpression in metastatic murine squamous carcinoma cells (12) and by findings from our laboratory indicating that ped/pea-15 is overexpressed in a number of human tumor cell lines (Formisano P. Perruolo G, and Beguinot F, unpublished observations). In addition, more recent work demonstrated that transgenic mice overexpressing ped/pea-15 feature increased occurrence of skin papillomas upon sequential treatment with the 7,12-dimethylbenz[a]anthracene (DMBA) tumor initiator and the TPA promoter (16), whereas the reverse occurs in ped/pea-15-null mice. The mechanisms responsible for ped/pea-15 overexpression in tumors and how it affects susceptibility to carcinogenesis are currently under investigation (see below).

Formisano et al. (16) demonstrated that TPA alone is sufficient to cause a rapid and sustained increase in ped/pea-15 expression in mouse skin, suggesting that ped/pea-15 overexpression is an early abnormality in chemically induced skin carcinogenesis in the rodent model. Indeed, further observations revealed that, in cell lines derived from different stages during DMBA/TPA-induced mouse skin tumor (72), the overexpression of ped/pea-15 occurs in the early phases of tumor promotion. Whether ped/pea-15 overexpression cooperates with other mechanisms responsible for tumor development or whether it is sufficient to induce transforming properties to the cells is presently being investigated. However, importantly, ped/pea-15 transgenic mice treated with DMBA/TPA also develop squamous cell carcinomas significantly more often than control mice, and those tumors more often progress to invasive spindle cell carcinomas in the transgenics (16), indicating that ped/pea-15 may also have a role in skin tumor progression, invasion, and metastasis. Studies performed by Glading et al. (20) showed that PED/PEA-15 inhibits invasion by tumor cells. Consistently, microarray investigation of breast cancer progression revealed that PED/PEA-15 protein expression levels inversely correlate with the invasive behavior of breast cancer, possibly due to reduced cellular motility (48). Moreover, women with high PED/PEA-15-expressing tumors survive longer than those with low PED/PEA-15-expressing tumors. Evidence obtained in human ovarian cancer also indicated that PED/PEA-15 antitumor activity is, in part, mediated by the induction of autophagy involving activation of the ERK1/2 pathway (3). Thus, although the antiapoptotic and oncogenic function of PED/PEA-15 has been well documented, PED/PEA-15 may also inhibit invasion. Some of the differences in the effect mediated by PED/PEA-15 could depend on the phosphorylation state or on the cellular context, which may be important in determining whether PEA-15 regulates cell survival or apoptosis. Further investigation in this area is expected to generate novel and valuable information for predicting individual risk to common cancers and for identifying novel targets for prevention and therapy of these cancers as well.

The Role of PED/PEA-15 in Glucose Tolerance and in Type 2 Diabetes

Type 2 diabetes is a complex heterogeneous group of metabolic conditions characterized by elevated levels of serum glucose and resulting from defects in both insulin secretion and insulin sensitivity. It is associated with an increased and premature risk of cardiovascular disease as well as specific microvascular complications, including retinopathy, nephropathy, and neuropathy (51). Type 2 diabetes pathogenesis results from interactions of a number of genes with environmental factors such as obesity, age, and nutrition. It is well established that type 2 diabetes has a polygenic nature, but in most affected patients, the gene(s) causing susceptibility to type 2 diabetes is still unknown. Very recent studies aimed at identifying common genetic variants associated with type 2 diabetes and at understanding their significance to and their role in the disease. This effort led to the identification of a number of diabetes susceptibility loci, which have been widely replicated in different and large populations (34, 44, 70).

In expression profile studies, PED/PEA-15 emerged as a gene overexpressed in adipose and skeletal muscle tissues and in skin fibroblasts from type 2 diabetic individuals independently of obesity (9). The molecular mechanisms of PED/PEA-15 overexpression are still unclear, but studies are in progress to investigate the mechanisms that may lead to dysregulating the expression of this gene in type 2 diabetic patients and in their first-degree relatives. The initial studies studies by Condorelli et al. (9) prompted further work to assess the significance of PED/PEA-15 to glucose metabolism and glucose tolerance. It has been shown that PED/PEA-15 overexpression represents a common trait among type 2 diabetics, because approximately one-third of the individuals diagnosed with the disorder exhibit PED/PEA-15 expression levels higher than 2 SD above the mean in the control subjects (61). In this same report, euglycemic offsprings of type 2 diabetics were also shown to express PED/PEA-15 levels comparable with those evidenced in their diabetic ancestors. These offsprings are known to be at very high genetic risk of type 2 diabetes (28), indicating that PED/PEA-15 overexpression is an early abnormality in the natural history of this type of diabetes. Two forms of further evidence supported the possibility that the overexpression of PED/PEA-15 in type 2 diabetes is, at least in part, genetically determined. First, the overexpression persists in skin fibroblasts upon many generations in culture (9). Second, our preliminary evidence indicates that PED/PEA-15...
polymorphisms are associated with increased gene expression in the Finnish population. However, whether the overexpression occurring in type 2 diabetic individuals is determined exclusively by genetic mechanisms or whether nongenetic mechanisms may also contribute to the overexpression and why PED/PEA-15 has not emerged in the recently published genome-wide association studies (70) are issues that still remain to be conclusively established.

Functional analysis in cultured skeletal muscle and adipose cells demonstrated that increased expression of PED/PEA-15 causes resistance to insulin action in glucose uptake (9). This abnormality depends largely on PED/PEA-15-induced dysfunction of the PKC signaling system. Indeed, studies published over the past years found evidence that PED/PEA-15 binds phospholipase D (PLD) isoforms, increasing their stability and intracellular diacylglycerol levels (71), thereby activating the dyacylglycerol-sensitive PKCα isoform. These studies also revealed that the active PKCα in PED/PEA-15-overexpressing cells and tissues prevents insulin induction of the PKCζ isoform. This is distinct from α and serves as a major activator of glucose transporter 4 (GLUT4) vesicle translocation toward the plasma membrane. Thus, studies in isolated muscle and adipose cells have shown that the overexpression of PED/PEA-15 may determine peripheral resistance to insulin action by dysregulating the signaling of PKC (Fig. 3) (10).

To assess the consequences of PED/PEA-15 overexpression on glucose tolerance, Vigliotta et al. (62) generated transgenic mice overexpressing ped/pea-15 ubiquitously, because this same situation occurs in many individuals with type 2 diabetes. Phenotyping these mice revealed that overexpression of PED/PEA-15 impairs glucose tolerance and may lead to diabetes under appropriate environmental conditions. Indeed, upon high-fat diet feeding, transgenic mice become overtly diabetic. The impaired glucose tolerance observed in the PED/PEA-15 transgenic mice was accompanied by significant resistance to insulin action on glucose disposal, reduced insulin-stimulated glucose transport in fat and skeletal muscles, and inhibition of insulin effect on GLUT4 membrane translocation. The mechanistic significance of these findings has been further corroborated by additional studies in ped/pea-15-null mice. In these animals, ablation of even a single copy of the ped/pea-15 gene causes a remarkable increase in insulin sensitivity, which is paralleled by enhanced PKCζ activity upon feeding. Thus, the additional concept has emerged that the control of insulin sensitivity is a physiological function of the PED/PEA-15 gene.

These observations in mice have profound implications for humans at increased risk of type 2 diabetes. Indeed, in nondiabetic offsprings of type 2 diabetics from the EUGENE2 cohort (30), PED/PEA-15 expression levels strongly correlate with insulin resistance in glucose disposal by the lean mass, as evidenced by euglycemic hyperinsulimemic clamp studies (61). These additional findings support the concept that PED/PEA-15 gene abnormalities provide a strong contribution to insulin resistance in these at-risk individuals. Whether in these same individuals genetic variation at the PED/PEA-15 locus associates with high PED/PEA-15 expression and insulin resistance is being investigated.

Phenotyping of the PED/PEA-15 transgensics provided initial evidence that insulin-resistance is not the only trait caused by overexpression of PED/PEA-15. Indeed, in these mice, insulin resistance in peripheral tissues was not sufficient to

Fig. 3. PED/PEA-15’s role in glucose metabolism. PED/PEA-15 binds PLD1 and enhances its stability and activity. The consequent increase of PKCα activation leads to the inhibition of PKCζ stimulation of glucose transporter 4 (GLUT4) translocation and impairs insulin-induced glucose uptake. IR, insulin receptor.
cause the observed abnormalities in glucose tolerance, leading to the hypothesis that PED/PEA-15-induced β-cell dysfunction might play an even greater role. This hypothesis has been supported by findings in an additional transgenic mouse overexpressing PED/PEA-15 selectively in pancreatic β-cells (35). Indeed, the β-cell-specific transgensics featured abnormalities in glucose tolerance that are almost superimposable to those occurring in mice that ubiquitously overexpress PED/PEA-15, indicating that β-cell overexpression is sufficient to impair glucose tolerance. Further studies in the different PED/PEA-15 transgenic and null mice enlightened the molecular mechanisms through which PED/PEA-15 affects β-cell function. First, it emerged that PED/PEA-15 restrains glucose-induced insulin secretion response in vivo. This action was also evidenced in islets from the PED/PEA-15 transgenic and null mice and in cultured β-cell lines where PED/PEA-15 gene was either overexpressed or silenced. Interestingly, both islets from PED/PEA-15-overexpressing mice and cultured MIN6 β-cells transfected with a PED/PEA-15 cDNA exhibited reduced insulin secretion response to glucose and arginine, both of which increase the ATP/ADP ratio but are fully responsive to potassium-induced insulin release. This initial finding suggested an action of PED/PEA-15 in the mechanism involved in the potassium rather than in those involved in calcium channel function. Indeed, subsequent gene profiling led to the discovery that the expression of the Sur1 and Kir6.2 potassium channel subunits is depressed in β-cells expressing high levels of PED/PEA-15 (35). Expression of the hepatic nuclear factor (HNF)-4β transcription factor was also decreased in these cells. More recent experiments clarified that, in β-cells expressing high levels of PED/PEA-15, the PKCα-dependent block of glucose-induced PKCζ impairs the phosphorylation of HNF-3β, thereby decreasing the expression of the Kir6.2 and the Sur1 potassium channel subunits. This, in turn, prevents the further increase in calcium flux otherwise triggered by glucose and inhibits the surge in calcium-dependent kinase activity necessary to drive insulin exocytosis and secretion in response to increasing glucose concentration. Thus, these studies have indicated that increased PED/PEA-15 expression may simultaneously contribute to multiple traits of the type 2 diabetes phenotype.

Understanding the physiological role of PED/PEA-15 in glucose tolerance has been followed by further efforts aimed at generating novel molecules interfering with PED/PEA-15 function and featuring potential pharmacological activity. Since PED/PEA-15 interaction with PLD1 appears to represent the initial event leading to PED/PEA-15 effect on glucose disposal, efforts were devoted to the identification of molecules inhibiting PED/PEA-15-PLD1 interaction. The minimum PLD1 region required for PED/PEA-15 interaction has been identified by Zhang et al. (71) and termed D4. The D4 region is located at the PLD1 COOH terminus and contains one of the two phosphodiesterase domains responsible for PLD1 enzymatic activity. Surface plasmon resonance and ELISA-like assays show that PED/PEA-15 binds in vitro the D4 domain with high affinity (63, 64). In these same studies, a PED/PEA-15 peptide spanning residues 1–24 and termed PED-(1–24) was shown to compete with the PED/PEA-15-D4 recognition (63, 64). When loaded into L6 cells overexpressing PED/PEA-15 and into myocytes derived from PED/PEA-15-overexpressing transgenic mice, PED-(1–24) abrogates the PED/PEA-15-PLD1 interaction and reduces PKCα activity to levels similar to controls. Importantly, the peptide restored insulin-stimulated glucose uptake, and similar results were obtained by expression of the D4 domain in L6 cells overexpressing PED/PEA-15, indicating that targeting PED/PEA-15-PLD1 interaction may represent a novel strategy to improve sensitivity to insulin action (63).

Regulation of PED/PEA-15 Intracellular Levels

As outlined above, studies in cellular and animal models have demonstrated a cause-effect relationship between the overexpression of PED/PEA-15 and impaired insulin action, insulin secretion, and tumor development. Thus, clarifying the mechanism controlling PED/PEA-15 cellular levels is expected to generate further insight into the molecular bases of both diabetes and cancer.

Run-on experiments in cultured cells from type 2 diabetic subjects provided evidence that, at least in part, the overexpression of PED/PEA-15 observed in these subjects is caused by transcriptional abnormalities, prompting different groups of investigators to elucidate how the PED/PEA-15 gene is regulated (9). The transcription start site of the PED/PEA-15 gene has been mapped by Wolford et al. (67). Further studies by our group revealed that this gene lacks a TATA box and that the shortest 5′-flanking fragment spanning nucleotides −39/+58 contains all the elements necessary to achieve basal promoter activity (58). Interestingly, HNF-4α, a member of the steroid receptor class of transcription factors, was found to repress PED/PEA-15 transcription through a promoter site located between nucleotides −335 and −320. This site closely matches the HNF-4α responsive element (RE) found in other genes and the GGGGCA/AGGTCA consensus HNF-4α binding site (18). This same RE is also recognized by chicken ovalbumin upstream promoter transcription factor II (COUP-TFI), another member of the orphan receptor family (36). At variance with HNF-4α, RE binding by COUP-TFI activates PED/PEA-15, indicating that transcription of PED/PEA-15 gene is dependent upon the intracellular balance of these positive and negative regulatory factors (Fig. 4). Both HNF-4α and COUP-TFI are involved in the control of glucose homeostasis (23, 41). Point mutations in HNF-4α cause MODY-type diabetes (37) and may have a role in the development of more common forms of type 2 diabetes (21). Also, COUP-TFI regulates several genes involved in lipid and glucose metabolism (32) and affects glucose tolerance in mice (2). Thus, polymorphisms in HNF-4α and in COUP-TFI or in their binding region on the PED/PEA-15 promoter may affect PED/PEA-15 expression and increase susceptibility to type 2 diabetes. In addition, there is evidence that the function of COUP-TFI and HNF-4α depends upon the repertoire of coregulatory proteins (43). Their identification is presently in progress and expected to clarify the mechanisms linking PED/PEA-15 overexpression to the genes controlling its expression. Indeed, the overexpression of PED/PEA-15 might represent a molecular abnormality downstream of several different diabetes risk genes and might serve as an effector of these genes. PED/PEA-15 expression is also posttranslationally regulated. Indeed, recent studies in our laboratory have shown that chronic exposure to the tumor-promoting agent TPA upregulates PED/PEA-15 protein expression by controlling its proteasomal degradation (42). Interestingly, in these same studies
there was evidence that PKCζ and CaMKII activities are necessary to enable TPA-dependent phosphorylation of PED/PEA-15 at the Ser116 residue. This phosphorylation event prevents ubiquitylation and proteosomal targeting and induces PED/PEA-15 intracellular accumulation, thereby enhancing its antiapoptotic action and contributing to tumor formation. Consistently, PED/PEA-15 is also phosphorylated by Akt/PKB at Ser116, resulting in PED/PEA-15 stabilization and enhanced cell survival (Fig. 4) (56). Akt/PKB is upregulated in a number of human cancers (6). Thus, simultaneous increases in PED/PEA-15 cellular levels and Akt/PKB activity might function cooperatively in tumorigenesis and/or tumor progression in human cancers.

The serine protease Omi/HtrA2 is a further molecule involved in controlling PED/PEA-15 protein content in the cell (55). Loss-of-function mutations of Omi/HtrA2 have been associated with familial forms of Parkinson’s disease (54). Mice featuring genetically deficient Omi/HtrA2 function also exhibit a Parkinsonian phenotype (33), further supporting the cause-effect relationship between PED/PEA-15 levels and these neurological abnormalities. Increased ped/pea-15 astrocytic levels have also been found in other murine models of neurodegeneration (69). Thus, dysregulation of PED/PEA-15 expression may be associated with different neurological abnormalities. Intriguingly, different neurodegenerative and mental disorders have recently been shown to exhibit increased incidence in diabetic individuals (66). The molecular base of this observation is largely unknown at the present time, but PED/PEA-15 represents an attractive target to investigate the increasing prevalence of these debilitating disorders in human diabetes.

Conclusions and Perspectives

Over the past 10 years, PED/PEA-15 has emerged as a key regulator of cell growth and glucose metabolism. Changes in PED/PEA-15 expression were shown to play an important role in tumor onset or progression and modify sensitivity to antineoplastic agents. However, PED/PEA-15 expression is inversely correlated to the invasive behavior of breast cancer, and high PED/PEA-15 expression is associated with improved overall survival in women with ovarian cancer, suggesting that PED/PEA-15 may represent a prognostic marker in ovarian cancer. Moreover, PED/PEA-15 is overexpressed in type 2 diabetic subjects, it generates resistance to insulin in peripheral tissues, and it impairs glucose-stimulated insulin secretion in mice models, dysregulating PKC signaling. Thus, PED/PEA-15 represents a new potential therapeutic target. Modulation of its
expression and/or function might represent an innovative strategy for prevention and treatment of these diseases.

Clarification of the molecular mechanisms responsible for dysregulating PED/PEA-15 expression at the translational and posttranscriptional levels is necessary to identify molecular strategies to modulate PED/PEA-15 levels. Efforts aimed at identifying molecules capable of interfering with PED/PEA-15 function are ongoing. Such molecules may, for example, penetrate the cells and disrupt PED/PEA-15-PLD interaction, thereby improving insulin sensitivity of the glucose transport machinery and glucose tolerance. Also, identification of the shortest-interacting region of PED/PEA-15 with proapoptotic proteins might enable the modulation of PED/PEA-15 anti-apoptotic action, increasing sensitivity to antineoplastic agents.

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**REFERENCES**


