PEA-15 Phosphoprotein: A Potential Cancer Drug Target

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Cancer is the leading cause of death for persons under the age of 85[1]. There have been tremendous advances in recent years in our understanding of both the environmental causes of many cancers and the molecular perturbations from which they arise. As cancer is a collection of diseases many of these environmental and molecular causes may be more important for one form of the disease than another. A number of proteins have been identified as either potential cancer chemotherapy targets or as molecular markers of cancer progression. Because cancer can arise from a variety of molecular pathologies it is unlikely that there will be a single silver bullet that defeats all forms of the disease and it is important that we continue to identify potential molecular targets. I here describe one such new target called PEA-15 (and also named variously PED-15, MAT-1 or PED/PEA-15). PEA-15 can affect both cell survival and proliferation and is overexpressed in many cancer cells including those of breast cancer, glioma, and squamous carcinoma.

PEA-15 Structure and Function

PEA-15 was independently cloned using four very different approaches. It was originally identified by Chneiweiss and colleagues as a 15 kilodalton, **P**hosphoprotein that is **E**nriched in **A**strocytes (hence the name PEA-15)[2]. Dr. Chneiweiss went on to clone the cDNA corresponding to PEA-15 using biochemical methods. Alternatively Nandi and colleagues cloned a portion of the message encoding PEA-15 (which they then called MAT-1) by screening expression libraries for their ability to transform mouse mammary cells[3]. This provided the first suggestion that PEA-15 might affect oncogenesis. Subsequently Beguinot and colleagues used differential display methods to clone PEA-15 (which they then called PED-15) as a phosphoprotein that is overexpressed in muscle from patients with type II diabetes[4]. In this work they report that PEA-15 expression inhibits insulin-stimulated glucose transport and may therefore be involved in the development of type II diabetes. Finally, in collaboration with Mark Ginsberg and others, I isolated PEA-15 by using expression cloning to identify proteins that block H-Ras signaling[5]. H-Ras is mutated in many different tumors and I therefore expected that proteins that regulate H-Ras may also affect the development, progression or pathology of some cancer cells.

PEA-15 is most highly expressed in the nervous system with particularly high levels in astrocytes and neurons of the hippocampus[6]. It is also expressed in lymphocytes and cell lines derived from gliomas, astrocytomas, and breast cancers. Indeed PEA-15 is reported to be overexpressed in some gliomas and breast cancer cell lines compared to normal cells from these tissues[7-9]. PEA-15 is phosphorylated at two sites by kinases including Protein Kinase C (serine 104), calcium calmodulin kinase II, and Akt (both at serine 116). The message is alternatively spliced in the non-coding region (3'UTR) and ubiquitously expressed in every tissue examined thus far[10]. However protein expression appears to be more confined. This suggests that PEA-15 translation may be regulated. Structurally PEA-15 consists of a death effector domain (DED) that corresponds to the first 80 of its 130 amino acids (Figure 1). These DEDs have been associated with proteins that regulate programmed cell death or apoptosis[11].

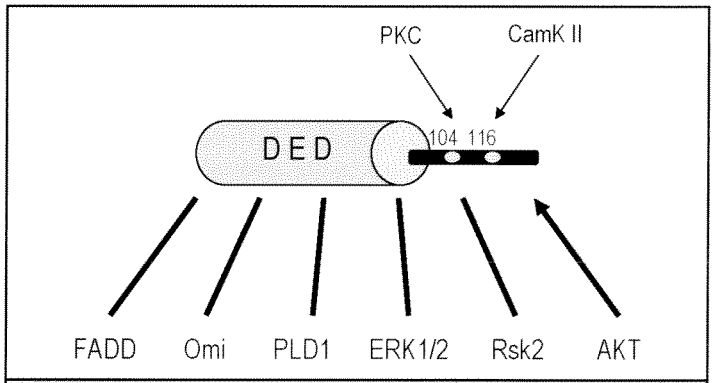

Figure 1.— PEA-15 Structure and Binding Partners

A schematic of PEA-15 is depicted. The protein is 130 amino acids in length and consists of a Death Effector Domain (DED) that constitutes amino acids 1 to 80. The DED is a homotypic binding domain found primarily in proteins that control extrinsic apoptosis. PEA-15 is phosphorylated at two serines. It is phosphorylated at serine 104 by protein kinase C (PKC) and at serine 116 by either Calcium calmodulin kinase II (CamK II) or AKT. These phosphorylations are depicted by arrows. PEA-15 binding partners are shown by connecting lines.

PEA-15 Binding Partners

PEA-15 is reported to bind several distinct proteins. These include proteins that regulate apoptosis, transcription, and proliferation. PEA-15 is reported to bind to FADD via its DED[12,13]. FADD is a pro-apoptotic linker protein that activates apoptosis by binding to the Fas receptor via its Death Domain (DD) at the plasma membrane in response to receptor binding to ligand[14]. This results in FADD recruitment, aggregation and activation of caspases 8 or 10. The

auto-activated caspases then cleave and activate other caspases that cause the death of the cell by degrading cellular proteins. PEA-15 binding to FADD is reported to block the ability of FADD to recruit and aggregate the caspases. Two of these reports showed direct binding of PEA-15 to FADD and/ or caspase 8[12, 13] while one report found no such interaction[15]. Subsequently, recruitment to FADD in the death initiation signaling complex (DISC) was shown to require phosphorylation of PEA-15 and to be affected by PKC phosphorylation at serine 104 [8, 9]. It remains to be determined if phosphorylation of PEA-15 alters its binding to FADD, however this is strongly suggested. Hence the discrepancy in the literature may be due to differences in the phosphorylation state of PEA-15.

Akt is a serine/threonine kinase that transduces both proliferative and survival signals. Akt binds to PEA-15 and phosphorylates it at serine 116[16]. Moreover phosphorylation at this site increases the anti-apoptotic action of PEA-15 perhaps by increasing its recruitment to the DISC. Omi/HtrA2 is a pro-apoptotic mitochondrial serine protease[17] that binds the DED of PEA-15[18]. It is reported to promote apoptosis upon release from the mitochondria in part by reducing cellular levels of PEA-15 and hence removing its anti-apoptotic influence. Omi/HtrA2 also acts by degrading other proteins called inhibitors of apoptosis (IAPs) such as XIAP[17]. Omi/HtrA2 is additionally proposed to be involved in cancer as a result of its role in apoptosis[19].

PEA-15 also binds to the MAP kinase ERK1/2[20] at the Carboxy-Terminal tail with some contribution by amino acids of the DED[21]. ERK has multiple substrates and can regulate transcription, proliferation, apoptosis and cell adhesion[22]. PEA-15 binds the MAP kinase insert region of ERK[21,23]. This is a region that varies significantly between different MAP kinases and this likely explains why PEA-15 does not bind other MAP kinases. PEA-15 binding to ERK retains ERK in the cytoplasm even upon activation of ERK. Many ERK substrates are nuclear such as the transcription factor ELK-1 and ERK nuclear translocation is required for ERK-dependent transcription, for example. Hence PEA-15 retention of ERK in the cytoplasm potentially alters the transcriptional fingerprint of cells stimulated by growth factors such as EGF. Indeed we have reported that overexpression of PEA-15 blocks ERK activation of ELK-1 and can also block CREB mediated transcription. Two separate mechanisms by which PEA-15 maintains ERK in the cytoplasm have been proposed. One is that PEA-15 has a nuclear export signal (NES) and can bind both active and inactive ERK and carry them out of the nucleus. A second is that PEA-15 binding to ERK interferes with ERKs' ability to bind to the nucleoporins of nuclear pore complex and enter the nucleus[23]. It remains unclear which is the predominant mechanism. Knockout of PEA-15 causes increased ERK localization to the nucleus and results in increased proliferation in astrocytes. Re-expression of PEA-15 returns proliferation to normal levels[20]. PEA-15 influence of proliferation provides a direct way by which PEA-15 could modulate cancer cell proliferation and tumor growth.

Rsk2 is a substrate of ERK and a kinase that can activate transcription and regulate apoptosis[24]. It contains two kinase domains and can also bind directly to ERK at its carboxyl terminal sequence. Rsk2 is suggested to bind solely to the Carboxyl-terminal tail of PEA-15 but the exact site is not yet determined[25]. PEA-15 overexpression affects Rsk2 in much the same way as it does ERK. It prevents Rsk2 translocation or accumulation in the nucleus and perhaps as a result blocks Rsk2-dependent activation of the transcription factor CREB[25]. PEA-15 appears to bind equally well to both active and inactive Rsk2. It is enticing to suggest that since Rsk2 is a substrate for ERK and that PEA-15 binds to both, that PEA-15 may enhance or impair ERK activation of Rsk2. Rsk2 is also reported to enhance activation of estrogen receptor alpha[26] and this may provide a mechanism whereby PEA-15 could influence breast cancer progression. Finally PEA-15 also can bind phospholipase D1 and D2 (PLD) and stabilize their expression[27]. PLD is a membrane associated lipase that catalyzes the production of phosphatidic acid and is reported to be involved in many signaling processes including ERK activation, apoptosis and glucose transport[28,29]. PLD binds to PEA-15 at a site consisting of sequence from both the DED and the carboxyl-terminal tail[27]. Co-expression of PEA15 with PLD1 increased PLD1 expression levels and activity. It is intriguing that PLD functions so closely resemble those of PEA-15. This may indicate a heretofore undiscovered interdependence of these proteins.

Therefore PEA-15 interacts with a broad array of proteins that can be generally grouped as either those involved in apoptosis, those involved in transcription, or those involved in both. Indeed the one common function attributed to all these proteins is that they regulate cell survival. Another protein with similarly diverse interactions is 14-3-3. 14-3-3 acts in part as a kind of protein sink, holding some proteins inactive until they are needed[30]. It is possible that PEA-15 may perform an analogous function, blocking some activities of its binding partners when expressed. It is therefore very important to determine how these interactions may be regulated.

Evidence of a role for PEA-15 in Oncogenesis

The first evidence that PEA-15 might play some role in cancer was found when it was identified as a mouse mammary transforming gene[3]. In these experiments a cDNA library was derived from mouse mammary tumors induced by lithium. The cDNA library was then screened for clones that neoplastically transformed a culture cell line (NIH 3T3). They showed that PEA-15 is normally expressed in mammary cells and that it was overexpressed in their lithium induced tumors. These investigators subsequently identified the Human ortholog of PEA-15 and demonstrated that in three human breast cancer cell lines (BT-10, T47-D, and MDA-MB-231) PEA-15 message showed a 10-fold overexpression compared to primary normal human breast epithelial cells[31,32]. However the open reading frame they initially focused on was a short region of the mRNA that is downstream of the region encoding PEA-15. We independently confirmed that MAT1 mRNA does cause transformation however in the same experiments when we express an mRNA containing only the coding region of PEA-15, we see no transformation (data not shown). Hence the transforming capability must reside in the large and highly conserved 3' UTR. Nandi and colleagues further showed that the PEA-15 mRNA is extremely stable and that there are differences in the expression of the alternatively spliced isoforms of the PEA-15 mRNA between mouse mammary epithelial cell lines[31]. They therefore propose that the 3'UTR may be a riboregulator in mammary tumorigenesis. This hypothesis has yet to be tested.

An alternative series of experiments by Hao and colleagues suggest that PEA-15 may be important in glioma cells. They found that glioma cell lines resistant to TRAIL induced apoptosis expressed 2

fold more PEA-15 than TRAIL sensitive lines[8]. Moreover, transfection of PEA-15 into the TRAIL sensitive lines rendered them resistant to apoptosis. Reducing PEA-15 levels in TRAIL resistant lines similarly rendered them sensitive to TRAIL induced apoptosis. These data support the hypothesis that PEA-15 prevents TRAIL induced apoptosis in these glioma cell lines. This anti-apoptotic function was dependent on PKC phosphorylation. These results are reminiscent of those of Chneiweiss and colleagues who found that astrocytes derived from mice lacking the PEA-15 gene were more sensitive to TNF-induced apoptosis than astrocytes from normal wild type mice [13]. It was subsequently reported that only PEA-15 phosphorylated at both serines could be recruited to the death inducing signaling complex at the plasma membrane where it could function to prevent apoptosis [9]. These results provide hope that targeting PEA-15 in gliomas (and perhaps other cancers) may render the cancer cells more sensitive to elimination when combined with other chemotherapies.

There have been other findings that also suggest PEA-15 may be important in the development or progression of some cancers. Studies using differential gene expression to identify changes in gene expression in a squamous carcinoma model found that PEA-15 was one of the genes that is upregulated[33]. Finally, PEA-15 has been reported to block signaling from the known oncogene Ras[5,7] and to promote Ras-mediated senescence[34]. Since Ras transforms cells and senescence prevents the transforming effect these studies support the possibility that PEA-15 can act as a tumor suppressor. This hypothesis is in conflict with that described above. How can PEA-15 both suppress tumorigenesis by blocking Ras and enhance tumorigenesis by blocking apoptosis. We do not yet have an answer to this question. It may be that whether PEA-15 acts as a tumor suppressor or tumor promoter depends on whether Ras activity is altered in a given cancer cell. It is important to address this issue in determining whether PEA-15 is a viable drug target.

Conclusions

PEA-15 is reported to prevent cell death[12,13,15], limit cell proliferation[20], alter transcription[20,25,27], and activate cell adhesion[5]. All of these events have been implicated in the formation, progression and metastasis of tumors. PEA-15 may therefore be a nexus at which multiple cancer cell pathologies can be targeted. Moreover since PEA-15 has a very limited expression profile but is overexpressed in many tumors it may be an ideal target for drug design.

For more information on the Cancer Research Center of Hawaii, please visit our web site at www.crch.org.

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