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Astrocyte Elevated Gene-1 (AEG-1): a multifunctional regulator of normal and abnormal physiology

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Abstract

Since its initial identification and cloning in 2002, Astrocyte Elevated Gene-1 (AEG-1), also known as metadherin (MTDH), 3D3 and LYsine-RIch CEACAM1 co-isolated (LYRIC), has emerged as an important oncogene that is overexpressed in all cancers analyzed so far. Examination of a large cohort of patient samples representing diverse cancer indications has revealed progressive increase in AEG-1 expression with stages and grades of the disease and an inverse relationship between AEG-1 expression level and patient prognosis. AEG-1 functions as a *bona fide* oncogene by promoting transformation. In addition, it plays a significant role in invasion, metastasis, angiogenesis and chemoresistance, all important hallmarks of an aggressive cancer. AEG-1 is also implicated in diverse physiological and pathological processes, such as development, inflammation, neurodegeneration, migraine and Huntington disease. AEG-1 is a highly basic protein with a transmembrane domain and multiple nuclear localization signals and it is present in the cell membrane, cytoplasm, nucleus, nucleolus and endoplasmic reticulum. In each location, AEG-1 interacts with specific proteins thereby modulating diverse intracellular processes the combination of which contributes to its pleiotrophic properties. The present review provides a snapshot of the current literature along with future perspectives on this unique molecule.

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1. Introduction

Over the past several decades, outstanding advances have enhanced our understanding of the molecular pathogenesis of cancer leading to the development of novel modalities of therapy. Despite these noteworthy achievements cancer remains the second leading cause of death in the United States (Jemal et al., 2010). A significant number of factors present major obstacles to developing therapies that might have lasting impact on the course of this fatal disease. Cancer involves a multi-step process from benign hyperplasia to metastatic tumors and is characterized by a combination of diverse genetic and epigenetic alterations in oncogenes, tumor suppressor genes, and genome-stabilizing genes (Vogelstein & Kinzler, 2004). However, most of the cancers exhibit the trait of 'oncogene addiction' where the survival of cancer cells depend on a specific oncogene, targeted inhibition of which impedes cell viability resulting in tumor regression (Weinstein, 2002). The in-depth understanding of the molecular etiology of carcinogenesis has identified these addictive oncogenes and led to the development of the novel concept of 'molecular targeted therapy' (Weinstein and Joe, 2006). Indeed, a number of targeted drugs have been identified and are being evaluated in clinical trials of cancer patients. Identification of an oncogene that is ubiquitously overexpressed in all or most cancers and plays a regulatory role in diverse and multiple processes of carcinogenesis might lead to the development of a '*pan-cancer*' therapy. Astrocyte elevated gene-1 (AEG-1) is now emerging as such an oncogene that may provide an ideal target to develop the next generation of effective cancer therapeutics.

AEG-1, originally identified as a neuropathology-associated gene in primary human fetal astrocytes (PHFA) (Su et al., 2002), is now established as an oncogene in a variety of cancers (Brown & Ruoslahti, 2004; Chen et al., 2010; Hu et al., 2009; Emdad et al., 2010; Lee et al., 2009; Li et al., 2008; Song et al., 2009; Song et al., 2010; Xia et al., 2010; Xu et al., 2010; Yoo et al., 2009a; Yu et al., 2009). In this review, we describe first, the historical perspective of AEG-1 including gene identification, and structural features; second, the evaluation of AEG-1 as a novel diagnostic/prognostic biomarker for a variety of cancers; and third, the understanding of the molecular and biochemical basis of oncogenic properties of AEG-1. We also briefly outline the potential role of AEG-1 in diverse diseases other than cancer, including neurodegeneration, inflammation and migraine.

2. Molecular cloning and structure of AEG-1

AEG-1 was initially identified in primary human fetal astrocytes (PHFA) as a novel gene induced by human immunodeficiency virus (HIV)-1 (Su et al., 2002, 2003). Subsequently, *in vivo* phage screening allowed the cloning of mouse AEG-1 as a protein mediating metastasis of breast cancer cells to lung and was named metadherin (Brown & Ruoslahti, 2004). The mouse/rat AEG-1 was also cloned as a tight junction protein named LYsine-RIch CEACAM1 co-isolated (LYRIC) (Britt et al., 2004) and by gene trapping techniques and was named 3D3/lyric (Sutherland et al., 2004). Using a novel modified RACE approach, the complete cDNA of AEG-1, containing 3611 bp, was cloned (Kang et al., 2005) of which 220 to 1968 bp sequence codes for AEG-1 protein with a predicted molecular mass of 64 kDa. The BLAST comparison indicated that AEG-1 gene has a unique structure with no similarity to currently known genes (Britt et al., 2004). AEG-1 homologues have been identified in other mammals with a high rate of identity (over 90%) and also in other

vertebrate species. However, AEG-1 homologues are not detected in lower invertebrates indicating that AEG-1 evolved to perform specialized functions in higher organisms.

Human AEG-1 gene is located in Chromosome 8q22 having 12 exons/11 introns. Chromosome 8q22 is known to be a hot spot for genomic alterations in several cancer cells involving HCC and breast cancer (Bergamaschi et al., 2006; Poon et al., 2006). Microarray and SNP array probing 250 Kb on either side of AEG-1 locus demonstrated increased copy number of AEG-1 in HCC (microarray, 32 of 91 tumors with a cut-off of >3; SNP, 36 of 103 tumors with a cut-off of >3), which was significantly correlated with expression level of AEG-1 (Yoo et al., 2009a). Fluoresence *in situ* hybridization (FISH) and genomic DNA quantitative PCR (qPCR) approaches also validated that 8q22 genomic gain was associated with increased expression of AEG-1 in breast cancer tissues (Hu et al., 2009).

Conceptual translational analysis using SMART and von Heijne's technique predicted that AEG-1 is a highly basic protein with a pI of 9.33 (von Heijne, 1992). AEG-1 has no known domains thus hampering prediction of its possible function based on structural analysis. Membrane protein structure prediction recognized AEG-1 as a single-transmembrane protein with a putative transmembrane domain (TMD) at the location between amino acid residues 51 to 72, which was further supported by independent prediction approaches such as PSORT II, TMpred, and HMMTOP (Sutherland et al., 2004) as well as immunofluorescence detection (Brown & Ruoslahti, 2004; Hu et al., 2009). AEG-1 contains three putative nuclear localization signals (NLS) between amino acids 79 to 91, 432 to 451, and 561–580 and AEG-1 is detected both in the nucleus as well as in the nucleolus (Sutherland et al, 2004; Thirkettle et al, 2009b). The COOH-terminal extended NLS-3 (a.a. 546–582) is the predominant regulator of nuclear localization, while extended NLS-1 (a.a. 78–130) regulates nucleolar localization (Thirkettle et al., 2009b). AEG-1 is also detected in the ER/nuclear envelop (Kang et al., 2005; Sutherland et al., 2004) in addition to its general localization in the cytoplasm. The region 378–440 a.a. harbors a putative lung-homing domain facilitating homing of metastatic breast cancer cells to the lung vasculature (Brown & Ruoslahti, 2004; Hu et al., 2009). There are a variety of putative post-translational modification residues and regulatory residues within the AEG-1 protein. The molecule contains a C-terminal '435-GALPTGKS-442' sequence, which is predicted to be a binding site of ATP/GTP. A variety of potential modification sites for phosphorylation on tyrosine, serine, and threonine amino acids are recognized in its conserved residues that might be phosphorylated by Protein Kinase C and A (PKC and PKA) pathways. AEG-1 in the extended NLS-2 (a.a. 415–486) undergoes monoubiquitination trapping it in the cytoplasmic compartment and it was shown that there is a redistribution of AEG-1 from cytoplasm to nucleus from benign prostatic hyperplasia to malignant prostate cancer (Thirkettle et al., 2009b).

3. Identification of AEG-1 as a potential diagnostic/prognostic marker for cancer

AEG-1 mRNA is ubiquitously expressed in all normal tissues, with higher expression detected in skeletal muscle and heart and in endocrine glands such as thyroid and adrenal gland (Kang et al., 2005). In cancer, AEG-1 is markedly overexpressed in all cancer indications studied so far, including HCC, breast, prostate, gastric, renal and colorectal cancer, non-small cell lung cancer (NSCLC), esophageal squamous cell carcinoma (ESSC), melanoma, glioblastoma multiforme (GBM), neuroblastoma and oligodendroglioma, compared to normal cells and matched non-neoplastic regions (Chen et al., 2010; Emdad et al., 2010; Kang et al., 2005; Lee et al., 2009; Li et al., 2008; Liu et al., 2010; Sarkar et al., 2009; Song et al., 2009; Song et al., 2010; Thirkettle et al., 2009b; Xia et al., 2010; Xu et al., 2010; Yoo et al., 2009a; Yu et al., 2009). Comparative expression analysis between patient

samples and matched non-neoplastic samples has established AEG-1 as a potential diagnostic/prognostic marker for cancer. In 225 clinical samples of breast cancer, immunohistochemical analysis detected AEG-1 expression in 93.3% cases with a significant increase in 44.4% cases having primary as well as metastatic breast cancer (Li et al., 2008). AEG-1 expression showed significant statistical correlation with the advanced stages of breast cancer and TNM classifications. In HCC patients, AEG-1 expression level correlates with the stages of the disease as well as grades of differentiation (Yoo et al., 2009a). Of the 109 HCC patients, overexpression of AEG-1 was detected in 102 cases (93.58%). The association between AEG-1 expression and HCC was further confirmed by analyzing AEG-1 mRNA expression using Affymatrix U133 plus 2.0 chips in 132 cases with various clinico-pathological stages of liver diseases including normal or non-neoplatic matched liver tissues (n = 10), cirrhotic tissues (n = 13), low-grade dysplastic nodules (n = 10), high-grade dysplastic nodules (n = 8), and HCC (n = 91). A significant overexpression of AEG-1 was detected in HCV-related HCC patients, compared to normal liver and cirrhotic tissues. Immunohistochemical staining of AEG-1 was analyzed in 20 prostate cancer (PC) and 20 benign prostatic hyperplasia (BPH) cases (Kikuno et al., 2007). Positive staining of AEG-1 was present in areas including small glandular structures. Statistically, intense AEG-1 staining (score +2) was observed in 16 out of 20 (80%) and significantly little (Score +1) to no staining (Score 0) were observed in only 4 cases (20%) of PC, compared to 90% of significantly little or no staining and 10% positive staining in BPH tissues. In another independent study immunohistochemical analysis of prostate tissue microarray (TMA) containing 143 PC cases and 63 BPH cases was performed to look into AEG-1 expression and distribution (Thirkettle et al., 2009b). When compared to BPH tissues, high level of AEG-1 staining strongly correlated with tumor malignancy, which was monitored using Gleason scores classified into three groups with low, moderate and high grades. AEG-1 predominantly displayed localization in nucleus of BPH tissues as well as in thyroid, and lung, while predominantly cytoplasmic expression was detected in PC. Interestingly, in 11 prostate bone metastases 9 (81.8%) patient showed increased staining of AEG-1 compared to normal bone indicating a role of AEG-1 in metastasis. In esophageal squamous cell carcinoma (ESSC) 80 out of 168 patient samples (47.6%) revealed increased expression of AEG-1 (Yu et al., 2009). The survival data of ESCC patients for 5 years were 40.7% in low AEG-1 expression group (n=88, 95% confidence interval, 0.5095 to 0.3044) and 22.6% in high AEG-1 expression group (n=80, confidence interval, 0.3177 to 0.1343). The clinical importance of AEG-1 was also demonstrated in non-small cell lung cancer (NSCLC) where 99 out of 200 (49.5%) patient samples showed highly expressed AEG-1 protein, which statistically correlated with clinico-pathological characteristics of NSCLC (Song et al., 2009). The increased expression of AEG-1 was observed in 6 out of 10 neuroblastoma patient samples compared to normal peripheral nerve tissues, normal astrocytes, and immortalized melanocytes (Lee et al., 2009). Two independent recent studies compared AEG-1 expression in glioblastoma multiforme (GBM) and other brain cancer patient samples with that in normal brain tissues (Emdad et al., 2010, Liu et al., 2010). Western blot analysis of AEG-1 in 9 normal brain, 25 GBM, 18 astrocytoma, 18 meningioma, 19 oligodendroglioma and 18 other types of brain cancer revealed 3 to 10-fold overexpression in brain cancer in >90% cases (Emdad et al., 2010). Immunohistochemical analysis detected positive AEG-1 staining in 265 out of 296 (89.5%) GBM patients among which 143 (48.3%) displayed low-level while 153 (51.7%) showed high level staining (Liu et al., 2010). In oligodendroglioma 51 out of 75 (68.0%) clinical cases showed increased expression of AEG-1 compared to normal brain tissues (Xia et al., 2010). Association of AEG-1 with prognosis in gastric cancer was confirmed in gastric cancer (Xu et al., 2010). Increased expression of AEG-1 was detected in 66 out of 105 (62.9%) clinical samples. Patients with high-level of AEG-1 showed poor survival rate (28.78%, a mean of 23 months) than patients with low-level of AEG-1 (61.5%, a mean of 38 months). High expression of AEG-1 was observed in 82 out of 146 (56.94 %) colorectal carcinoma patients compared to normal

intestinal mucosa (0 of 45, 0%), low-grade adenoma (5 of 31, 13.89%), and high-grade adenoma (7 of 15, 46.67%) (Song et al., 2010). Immunohistochemical analysis also evaluated that AEG-1 protein is highly expressed in 96 out of 102 (94.1%) cases of renal cell carcinoma (RCC) patients (Chen et al., 2009). The high incidence of AEG-1 overexpression in diverse cancer patients with poor prognosis strongly suggests that AEG-1 might be employed as a universal diagnostic/prognostic marker for cancer.

4. Oncogenic functions of AEG-1

4.1. Effect of AEG-1 on proliferation and invasion

Normal cells traverse a step-wise progression of independence of growth control, immortalization transformation and finally acquisition of invasive and metastatic abilities (Fisher, 1984). In parallel with evaluation of AEG-1 as a biomarker for cancer, a substantial body of studies highlight the pathophysiological role of AEG-1 in mediating oncogenesis that involve alterations in cell growth and proliferation, anchorage-independent growth, migration, invasion, chemoresistance, angiogensis and *in vivo* tumorigenesis and metastasis. Manipulation of AEG-1 expression (ectopic overexpression or knockdown) showed that AEG-1 promotes proliferation in many cancer cells including HCC, ESCC, breast and prostate cancer, GBM and neuroblastoma (Yoo et al., 2009a; Yu C et al., 2009; Li et al., 2009; Lee et al., 2009). Knocking down AEG-1 induces apoptosis in prostate cancer and neuroblastoma cells (Kikuno et al., 2007; Lee et al., 2009). Overexpression of AEG-1 in normal human cells, such as immortal primary human fetal astrocytes (IM-PHFA) and normal immortal melanocytes (FM516-SV) protects them from serum starvation-induced apoptosis indicating that as an anti-apoptotic protein AEG-1 might function as an oncogene (Lee et al., 2008). Anchorage-independent growth in soft agar is a unique characteristic of transformed cells. IM-PHFA, FM-516-SV and normal cloned rat embryonic fibroblasts (CREF) acquire this ability upon forceful overexpression of AEG-1 which is further augmented in the presence of Ha-ras (Emdad et al., 2009; Kang et al., 2005; Lee et al., 2006). Knocking down AEG-1 significantly abrogated clonogenic activity conferred by Ha-ras indicating that AEG-1 plays a central role in mediating Ha-ras function (Lee et al., 2006). Indeed AEG-1 is a downstream target gene of Ha-ras that increases AEG-1 transcription by activating PI3K/Akt pathway leading to binding of c-Myc to AEG-1 promoter (Lee et al., 2006). Invasion through an artificial matrix such as Matrigel® is indicative of increased metastatic ability. Indeed, enhanced expression of AEG-1 increased Matrigel® invasive potential of HeLa, human HCC, neuroblastoma, malignant glioma cells and CREF (Emdad et al., 2006; Emdad et al., 2009; Lee et al., 2009; Sarkar et al., 2008; Yoo et al., 2009a).

4.2. AEG-1 and chemoresistance

Chemoresistance is an important hallmark of aggressive cancers. Recent findings suggest that AEG-1 contributes to broad-spectrum resistance to various chemotherapeutics including 5-fluorouracil (5-FU), doxorubicin, paclitaxel, cisplatin and 4-hydroxycyclophosphamide (4-HC) (Yoo et al., 2009a, 2009b, 2010; Hu et al., 2009; Liu et al., 2009). Gene expression profiles of AEG-1-overexpressed human HCC cells versus control cells identified several upregulated genes implicated in chemoresistance including drug-metabolizing enzymes, such as dihydropryimidine dehydrogenase (DPYD), cytochrome P450B6 (CYP2B6) and dyhydrodiol dehydrogenase (ARK1C2); ATP-binding cassette transporter ABCC 11/MRP8; and the transcription factor LSF/TFCP2 (Yoo et al., 2009a). 5-FU works by inhibiting thymidylate synthase (TS), an enzyme necessary for DNA synthesis, and LSF transcriptionally regulates TS expression (Longley et al., 2003; Powell et al., 2000). AEG-1 induces 5-FU resistance by increasing the expression of LSF and subsequently TS and also by increasing the catabolism of 5-FU by enhancing DPYD expression (Yoo et al., 2009b).

While transcriptional regulation by AEG-1 plays a role in mediating 5-FU resistance, augmentation of translation of multidrug resistance gene 1 (MDR1/ABCB1), the most common member of ABC-transporters, by AEG-1 confers resistance to doxorubicin in human HCC cells (Yoo et al., 2010). By activating PI3K/Akt pathway, AEG-1 facilitates association of MDR1 mRNA to polysomes resulting in accumulation of MDR1 protein and increased efflux of doxorubicin from the cells. In addition AEG-1 also causes a decrease in ubiquitin-proteosomal degradation of MDR1 protein (Yoo et al., 2010). In nude mice xenograft model using human HCC cells inhibition of AEG-1 by a lentivirus expressing siRNA significantly increased the anti-cancer effect of 5-FU and doxorubicin (Yoo et al., 2009b; Yoo et al., 2010). Through a comparison of gene expression profile between AEG-1 knockdown LM2 breast cancer cells versus control cells a number of additional chemoresistance-related genes involving aldehyde dehydrogenase 3A1 (ALDH3A1) and hepatocyte growth factor Receptor (HGFR/c-Met) were identified (Hu et al., 2009). Overexpression and siRNA knockdown studies revealed the importance of ALDH3A1 and c-Met in mediating AEG-1-induced resistance to paclitaxel, doxorubicin and 4-HC.

4.3. AEG-1 promotes angiogenesis and metastasis

The *in vitro* findings are amply supported by *in vivo* data using nude mice xenograft models. AEG-1 overexpression in human HCC cells resulted in highly aggressive, angiogenic and metastatic tumors while inhibition of AEG-1 abrogated subcutaneous tumor formation by human HCC and neuroblastoma cells and intracerebral tumor formation by human glioma cells (Yoo et al., 2009a, Lee et al., 2009, Emdad et al., 2010). AEG-1 overexpression in human HCC cells resulted in increased production of angiogenic factors, such as, vascular endothelial growth factor (VEGF), placental growth factor (PIGF) and fibroblast growth factor-α (FGFα) (Yoo et al., 2009a). Similarly, CREF-AEG-1 cells also generated highly aggressive and angiogenic tumors in nude mice (Emdad et al., 2009). The tumor sections revealed augmented expression of specific angiogenesis molecules including angiopoietin-1 (Ang1), matrix metalloprotease (MMP)-2 and hypoxia inducible factor (HIF)1-α (Emdad et al., 2009). The pro-angiogenic property of AEG-1 was also supported by chicken chorioallantoic membrane (CAM) assay and human umbilical vein endothelial cells (HUVEC) differentiation assay (Emdad et al., 2009). Overexpression of AEG-1 augmented *in vivo* metastasis of human breast cancer cells and HEK293T cells especially to the lungs, while inhibition of AEG-1 reversed this phenomenon for both human and mouse breast cancer cells (Brown & Ruoslahti, 2004; Hu et al., 2009). Animals injected with AEG-1-overexpressing breast cancer cells had decreased survival rate while AEG-1 inhibition increased the survival rate (Hu et al., 2009). Increased adhesion to endothelial cells conferred by AEG-1 has been postulated to be the mechanism of increased metastasis by AEG-1 (Hu et al., 2009). The AEG-1-interacting molecule on the endothelial cells remains to be identified.

5. Signaling pathways activated by AEG-1

AEG-1 is unique in its ability to profoundly modulate global gene expression changes leading to alterations in expression of genes regulating proliferation, invasion, chemoresistance, angiogenesis, senescence and metastasis (Yoo et al., 2009a; Hu et al., 2009). Additionally, AEG-1 also activates multiple pro-tumorigenic signal transduction pathways that play a major role in mediating the pleiotrophic functions of AEG-1.

5.1. Activation of phosphotidylinositol-3-kinase/AKT (PI3K/AKT) pathway

Activation of PI3K/AKT pathway plays a fundamental role in carcinogenesis. AEG-1 expression is not only induced by PI3K/AKT pathway and c-Myc, it itself activates PI3K/AKT and induces c-Myc expression, and in neuroblastoma cells N-Myc expression, thus

setting forth a vicious cycle of tumorigenic events (Lee et al., 2006; Lee et al., 2008; Lee et al., 2009). Activation of PI3K/AKT pathway facilitates AEG-1-induced protection of normal cells from serum-starvation-induced apoptosis via downregulation of pro-apoptotic Bad and p21 protiens and upregulation of MDM2 protein nullifying p53 function (Lee et al., 2008). AKT activation is also necessary for AEG-1-induced upregulation of HIF1-α in HUVEC and activation of VEGF promoter in human glioma cells (Emdad et al., 2009). Inhibition of AEG-1 in prostate cancer cells downregulates AKT activation and leads to upregulation of forkhead box (FOXO)3a activity and p27 resulting in apoptosis (Kikuno et al., 2007). In esophageal cancer cells, activation of AKT by AEG-1 leads to upregulation of cyclin D1 and downregulation of p27 (Yu et al., 2009). PI3K/AKT activation is also necessary for increased polysome association of MDR1 mRNA leading to chemoresistance (Yoo et al., 2010). Thus PI3K/AKT activation mediates multiple aspects of AEG-1 function. However, the mechanism by which AEG-1 activates this pathway remains to be determined.

5.2. Activation of Nuclear Factor-κB (NF-κ B) pathway

The second major signaling pathway activated by AEG-1 is NF-κB, a transcription factor playing a crucial role in carcinogenesis. The association between AEG-1 and NF-κB pathway was first demonstrated in HeLa cells and human glioma cells (Emdad et al., 2006; Sarkar et al., 2008). Ectopic expression of AEG-1 using a replication-incompetent adenovirus (Ad.AEG-1) in HeLa cells led to increased DNA binding of the transcriptional activator p50/p65 complex of NF-κB resulting in induction of NF-κB downstream targets including intercellular adhesion molecule (ICAM)-3, ICAM-2, selectin E, selectin P ligand, selectin L, toll-like receptor (TLR)-4, TLR-5, FOS, JUN, and IL-8 (Emdad et al., 2006). These proteins mediate NF-κB-induced proliferation, angiogenesis and inflammation, all required for the carcinogenic process indicating that activation of NF-κB also mediates multiple aspects of AEG-1 function. Inhibition of NF-κB pathway using an adenovirus expressing mt32IκBα superrepressor (Ad.IκBα-mt32) markedly reversed AEG-1-induced agar cloning efficiency and Matrigel® invasion in human glioma cells (Sarkar et al., 2008). Activation of NF-κB by AEG-1 has also been detected in human prostate and liver cancer cells (Kikuno et al., 2007; Yoo et al., 2009a). The mechanism of AEG-1-induced activation of NF-κB will be discussed in the next section.

5.3. Activation of mitogen activated protein kinase (MAPK) and Wnt pathways

AEG-1 activates mitogen activated protein kinase (MAPK) pathways, especially ERK and p38 MAPK, in human HCC cells that promotes increased invasion and anchorage-independent growth without significantly affecting cell proliferation (Yoo et al., 2009a). Interestingly, activation of ERK cross talks with Wnt signaling pathway thereby activating the latter. The Wnt/β-catenin pathway is an important pro-tumorigenic pathway for multiple cancers (Clevers, 2006). The transcription factor LEF-1, the final executor of Wnt pathway, needs to heterodimerize with β-catenin for its action. In the off-state, β-catenin is phosphorylated by GSK3α/β, which leads to ubiquitin-proteosome-mediated degradation of β-catenin, while in the on-state following binding of Wnt to Frizzled receptors (Fzd) inactivation of GSK3α/β by phosphorylation results in increased β-catenin level and nuclear translocation of β-catenin to activate gene transcription. Wnt pathway is activated by AEG-1 in multiple ways (Yoo et al., 2009a): (1) AEG-1 induces expression of LEF-1 itself as well as LEF-1-induced genes, such as c-Myc. (2) Activation of ERK by AEG-1 leads to GSK3α/β phosphorylation and inactivation resulting in nuclear translocation of β-catenin. (3) AEG-1 downregulates APC and CTBP2, the negative regulators of Wnt pathway. The importance of Wnt signaling in mediating AEG-1 action was demonstrated by inhibiting LEF-1 that resulted in significant abrogation of AEG-1-induced invasion of HCC cells.

6. AEG-1-interacting proteins contributing to tumorigenesis

Interaction with other proteins in multi-protein complexes is a predominant means by which AEG-1 exerts its multiplicity of functions. In each intracellular location AEG-1 interacts with specific protein(s) thus influencing diverse intracellular events. Although a potential binding partner of cell surface AEG-1, such as that might be present on pulmonary endothelial cells, remains to be identified, several intracellular interacting partners of AEG-1 have been identified that provides interesting insights into the mechanism of action of AEG-1.

6.1. NF-κB

As mentioned earlier activation of NF-κB signal pathway significantly contributes to AEG-1-mediated oncogenic events and AEG-1 activates NF-κB by directly interacting with the p65 subunit of NF-κB (Emdad et al., 2006; Sarkar et al., 2008). Tumor necrosis factor-α (TNF-α) is induced by HIV-1 infection and functions as a major activator of NF-κB. AEG-1 expression is induced by TNF-α and interestingly, treatment of HeLa cells or human glioma cells with TNF-α results in nuclear translocation of AEG-1 where it interacts with the p65 subunit of NF-κB (Emdad et al., 2006; Sarkar et al, 2008). Although AEG-1 does not directly bind to DNA, it interacts with NF-κB as well as with the transcription co-activator cyclic AMP-responsive element binding protein (CREB)-binding protein (CBP). Chromatin immunoprecipitation (ChIP) assays demonstrated that upon TNF-α treatment, AEG-1 facilitates the complex formation between NF-κB and CBP on the interleukin-8 (IL-8) promoter. Inhibition of AEG-1 by siRNA does not affect recruitment of NF-κB but precludes recruitment of CBP to the IL-8 promoter indicating that AEG-1 functions as a bridging factor between NF-κB and CBP bringing them together to the basal transcription machinery to induce IL-8 transcription (Sarkar et al., 2008). Deletion of the NH_2-terminal 71 a.a. residues blocks AEG-1-induced NF-κB activation, IL-8 expression as well as invasion and anchorage-independent growth of malignant glioma cells (Sarkar et al., 2008). However, this region does not interact with NF-κB, rather a.a. 101–205 region was identified as the p65-interacting domain of AEG-1. It might be possible that the proximal 71 a.a. region of AEG-1 interacts with CBP thereby mediating NF-κB activation.

6.2. PLZF

PLZF, a transcriptional repressor associated with growth suppression and apoptosis, was identified as an AEG-1 interacting protein using the yeast two-hybrid approach (Thirkettle et al., 2009a). Co-immunoprecipitation assays revealed that NH_2-terminal 1–285 a.a. and COOH-terminal 487–582 a.a. of AEG-1 interacted with PLZF. The region a.a. 322–404 of PLZF containing two lysine residues for activation of PLZF by SUMOylation is necessary for interaction with AEG-1 (Chao et al., 2007). Inhibition of PLZF SUMOylation by point mutation resulted in lack of interaction between AEG-1 and PLZF, indicating that interaction of PLZF with AEG-1 is dependent on the active status of PLZF (Thirkettle et al., 2009a). AEG-1-PLZF interaction was detected in nuclear bodies and it was shown that AEG-1 increased association of PLZF with histone deacetylase 4 (HDAC4) (Thirkettle et al., 2009a). PLZF is a suppressor of c-Myc transcription and ChIP assays documented that AEG-1 prevented recruitment of PLZF to the c-Myc promoter thereby increasing c-Myc transcription. PLZF neutralization thus might be another way, along with effects on the PI3K/AKT and Wnt signaling pathways, by which AEG-1 activates c-Myc. In these contexts, AEG-1 and c-Myc might have an intimate and supplementary role in mediating tumorigenesis.

6.3. BCCIPα

BCCIPα is a tumor suppressor that binds to p21 (*mda*-6/CIP-1) and enhances p21-mediated inhibition of Cdk2 kinase. Loss of BCCIPα impairs G_1/S checkpoint activation following DNA damage and in conjunction with BRCA2, BCCIPα plays a role in homologous recombination repair of DNA damage and contributes to maintenance of chromosome stability (Ono et al., 2000). Genomic profiling of primary glioma cell lines demonstrated that GBM progression is associated with reduced expression of BCCIPα (Roversi et al., 2006). Interaction of BCCIPα with BRCA2 leads to inhibition of growth in breast and brain cancer cells (Liu et al., 2001). Yeast two-hybrid screening identified BCCIPα as an AEG-1-Interacting protein in prostate cancer cells and it was demonstrated that a.a. 72–169 region of AEG-1 interacts with BCCIPα (Ash & Britt, 2008). Interaction of AEG-1 with BCCIPα leads to reduction of BCCIPα protein levels through increased proteosomal degradation of BCCIPα. However, the functional role of BCCIPα degradation in mediating AEG-1 function remains to be determined.

6.4. Staphylococcal nuclease domain containing 1 (SND1)

Two independent approaches, yeast two-hybrid screening using a human liver cDNA library, and isolation of AEG-1-interacting proteins by co-immunoprecipitation followed by identification by mass spectrometry, revealed SND1 as an AEG-1 interacting protein (Yoo et al., 2010, submitted for publication). SND1 is located both in the nucleus and cytoplasm. In the nucleus it facilitates transcription as a co-activator and mRNA splicing by interacting with the splicesome machinery (Yang et al, 2007). In the cytoplasm, it functions as a nuclease in the RNA-induced silencing complex (RISC) in which small RNAs (such as siRNAs or miRNAs) are complexed with ribonucleoproteins to ensue RNAi-mediated gene silencing (Caudy et al., 2003). It was demonstrated that AEG-1, via the region 101–205 a.a., interacts with SND1 in the cytoplasm and not in the nucleus and AEG-1 is also a component of RISC and is required for optimum RISC activity (Yoo et al., 2010, submitted for publication). Both AEG-1 and SND1 are overexpressed in HCC and RISC activity was found to be higher in human HCC cells compared to normal immortal hepatocytes. It was hypothesized that increased RISC activity might contribute to oncomiR-mediated degradation of tumor suppressor mRNAs thus facilitating hepatocarcinogenesis. Indeed, inhibition of AEG-1 or SND1 increased while overexpression of AEG-1 or SND1 decreased the mRNA level of the tumor suppressor PTEN, a target of miRNA-221 which is overexpressed in HCC. These studies reveal a novel function of AEG-1 in mediating RNAi-mediated gene silencing.

7. Potential involvement of AEG-1 in other physiological and pathological processes

7.1. Neurodegeneration

AEG-1 was originally identified as one of the 16 genes displaying elevated expression in primary human fetal astrocytes upon HIV-1 infection, treatment with HIV-1 glycoprotein gp120 or TNF-α, an inflammatory mediator of HIV-1 (Su et al., 2002, 2003). However, what role AEG-1 plays in HIV pathogenesis remains to be determined. Initial observations indicate that AEG-1 might play a role in HIV-activated dementia (HAD) by causing neuronal death. Glutamate is the main excitatory amino acid transmitter in the mammalian CNS and is probably involved in most aspects of normal brain function, including cognition, memory and learning (Fonnum, 1984). However, excessive glutamate exposure is toxic to neurons, probably resulting in large part from glutamate-triggered Ca^{2+} entry (Choi, 1988). Astroglial glutamate transporter (excitatory amino acid transporter-2/EAAT2) is responsible for the majority of the clearance of glutamate from neuronal synapses in the CNS. Impaired

glutamate uptake by glial cells can result in cell death from excessive levels of glutamate and overstimulation of glutamate receptors (Choi, 1988). Indeed, downregulation of EAAT2 followed by accumulation of glutamate in the extracellular fluid and neuronal death have been documented in a wide variety of neurodegenerative disorders, including amyotrophic lateral sclerosis (ALS), Alzheimer's disease, several forms of epilepsy, ischemia/stroke, HAD, traumatic brain injury and hepatic encephalopathy (Choi, 1988; Doble, 1999). It was documented that AEG-1 repressed EAAT2 promoter activity (Kang et al., 2005) indicating that overexpression of AEG-1 might lead to glutamate accumulation in the synapses and neurodegeneration.

7.2. Inflammation

NF-κB is a key regulator of pro-inflammatory cytokines and activation of NF-κB by AEG-1 indicates a potential role of AEG-1 in the inflammatory processes. A recent study demonstrates that in promonocytic cells AEG-1 is induced by lipopolysaccharides (LPS) via the NF-κB pathway (Khuda et al., 2009). However, AEG-1 is also required for LPS-induced activation of NF-κB and inhibition of AEG-1 abrogated LPS-mediated induction of TNFα or prostaglandin E2 production. AEG-1 was not induced by other toll-like receptor activators apart from LPS which activates TLR4. AEG-1 thus might be a target molecule for LPS-related diseases, such as septic shock and systemic inflammatory response syndrome.

7.3. Huntington disease (HD)

In transgenic mouse models of Huntington disease, regulator of ribosome synthesis 1 (Rrs1) is overexpressed and it is hypothesized that Rrs1 might contribute to HD pathogenesis by initiating ER stress (Fossale et al., 2002; Carnemolla et al., 2009). Interestingly, colocalization of Rrs1 with AEG-1 in the endoplasmic reticulum (ER) was observed suggesting that both Rrs1 and AEG-1 might contribute to HD-associated ER stress (Carnemolla et al., 2009). However, the functional role of AEG-1 in HD has not been experimentally documented.

7.4. Migraine

A genome-wide association study (GWAS) implicated AEG-1 in migraine, a neurological disorder with severe headache (Anttila et al., 2010). In 2,731 individuals it was identified that migraine is associated with the allele rs1835740 on chromosome 8q22, located between AEG-1 and plasma glutamate carboxypeptidase (PGCP). In expression quantitative trait locus analysis, the transcript level of AEG-1 significantly correlated to rs1835740 in lymphoblastoid cell lines (LCL), but not in fibroblasts and primary T cells. It was hypothesized that glutamate accumulation as a consequence of EAAT2 downregulation by AEG-1 and increased PGCP activity might increase susceptibility to migraine through increased sensitivity to cortical spreading depression, the likely mechanism for the migraine aura, as well as through glutamate involvement in central sensitization, the underlying mechanism of allodynia during a migraine attack.

7.5. Embryogenesis and development

Recent observations on the expression patterns of AEG-1 in staged mouse embryos indicate a potential involvement of AEG-1 in embryonic development (Jeon et al., 2010). By whole-mount immunohistochemistry the expression of AEG-1 was detected in dorsal midbrain and fronto-nasal process at E9.5 and in forelimb, hindlimb and pharyngeal arches at E9.5 to E10.5, suggesting that AEG-1 might contribute to differentiation and proliferation of progenitor cells at early stages of embryogenesis. The increased expression of AEG-1 at stages E12.5 to E18.5 was observed in brain, olfactory and skeletal systems, indicating a potential role of AEG-1 in the development of these organs. Colocalization of AEG-1 with

the proliferation marker Ki67 at specific stages and processes during embryo development suggested that the expression of AEG-1 might contribute to cell proliferation in embryogenesis. Analysis of the phenotype of an AEG-1 knock-out mouse will confirm these hypotheses deduced from expression analysis.

8. Current and developmental therapeutics impacting on AEG-1 function

Considering the fact that AEG-1 is overexpressed in a wide variety of cancers and it regulates fundamental processes of carcinogenesis, such as transformation, invasion, metastasis, angiogenesis and chemoresistance, inhibition of AEG-1 might be a very viable and effective anti-cancer strategy. The lack of any existing AEG-1 inhibitor prompts us to formulate potential therapeutic strategies based on drugs that are currently used or in development and that might impact AEG-1 function. Two major pathways activated by AEG-1 are NF-κB and PI3K/Akt. Activation of NF-κB depends upon ubiquitin-proteasome-mediated degradation of its inhibitor IκB that allows NF-κB to translocate into nucleus and regulate transcription. Proteasome inhibitors block IκB degradation thus preventing NF-κB activation (Navon & Ciechanover, 2009). Bortezomib, a proteasome inhibitor, has been approved by FDA as a drug for the treatment of multiple myeloma, a condition in which NF-κB is often constitutively activated (Kane et al., 2003). The successful entry of Bortezomib in the therapeutic arena facilitates its usage in other cancer indications as well. New generation of proteasome inhibitors, with higher efficacy than Bortezomib, are being developed although none of them have been approved by FDA yet (Navon & Ciechanover, 2009). A lot of efforts are being dedicated to developing PI3K/Akt inhibitors and there are at least nine different PI3K inhibitors that are being evaluated clinically and showing potential anti-cancer efficacy (http://clinicaltrials.gov) (Workman, et al., 2010). A combination of Bortezomib and PI3K inhibitor(s) might be one strategy to counteract cancer phenotypes induced by AEG-1.

9. Conclusion and future perspectives

Although significant progress has been made since its initial cloning in 2002, it is eminently clear that we have just begun to scratch the surface relative to our understanding of the diversity of activities that AEG-1 regulates in both normal and abnormal physiology. A growing body of literature continues to establish AEG-1 as a very important molecule regulating a variety of pathological and physiological processes by modulating transcription, translation, RNAi and signaling pathways. The observation that AEG-1 is able to interact with a variety of proteins and assist in the formation of multi-protein complexes indicates that AEG-1 might be a new member of scaffold proteins. The multiple functions of AEG-1 highlight several important clinical implications. AEG-1 might be a ubiquitous biomarker for aggressive cancers with potential for routine screening of patients. AEG-1 expression might also help stratify patients receiving chemotherapeutic regimens based on the broad-spectrum chemoresistance conferred by AEG-1. The cell surface AEG-1 might be used for diagnostic and therapeutic purposes. Poly(vinyl alcohol)-pyrene, a water soluble probe containing a fluorescent marker, was fused with anti-AEG-1 antibody (Medine et al., 2010). This complex efficiently bound to cells expressing high levels of AEG-1 on the surface that could be imaged by fluorescence microscopy. Although this strategy was evaluated only *in vitro*, it provides the basis for evaluation of similar approaches *in vivo*. Similarly, therapeutic toxins or radioactive isotopes might be conjugated with anti-AEG-1 antibody for targeted destruction of AEG-1 overexpressing cells. AEG-1 itself might be targeted not only for cancers but also for several other diseases, including septic shock, neurodegenerative disorders and migraine. Since routine RNAi-based treatment is in its infancy relative to clinical application, development of small molecule inhibitors of AEG-1 is mandatory, which is complicated by the fact that AEG-1 has no known domains or motifs that might be

suitable targets. However, protein-protein interaction studies reveal that the NH_2-terminal region, especially in the neighborhood of 100–205 a.a. of AEG-1, is the hot spot for potential interaction with other proteins. Solving the crystal structure of this novel protein-protein-interaction domain might help in the design of small molecule inhibitors of AEG-1. High throughput screening with small molecules that disrupt interaction of AEG-1 with its known interacting proteins might be another approach to identify AEG-1 inhibitors. Current and future studies focus on these translational aspects of AEG-1 as well as understanding AEG-1 function in physiological and pathological processes by using transgenic and knockout mouse models.

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Abbreviations

AEG-1	Astrocyte elevated gene-1
ALDH3A1	Aldehyde dehydrogenase 3A1
CAM	chorioallantoic membrane
CBP	Cyclic AMP-responsive element binding protein (CREB)-binding protein
ChIP	Chromatin immunoprecipitation
CREF	Cloned rat embryonic fibroblasts
EAAT2	Excitatory amino acid transporter 2
ER	Endoplasmic reticulum
ERK	Extracellular signal regulated kinase
FISH	Fluorescence in-situ hybridization
Fzd	Frizzled
5-FU	5-fluorouracil
GBM	Glioblastoma multiforme
HCC	Hepatocellular carcinoma
HDAC	Histone deacetylase
HGFR	Hepatocyte growth factor receptor
HIF	Hypoxia-inducible factor
HIV	Human immunodeficiency virus
HUVEC	Human umbilical vein endothelial cells
4-HC	4-hydroxycyclophosphamide
IL-8	Interleukin 8
LEF-1	Lymphoid enhancing factor-1
LYRIC	LYsine-RIch CEACAM1 co-isolated

MAPK	Mitogen activated protein kinase
MMP	Matrix metalloproteinase
MTDH	Metadherin
NF-κB	Nuclear Factor-κB
NLS	Nuclear localization signal
PHFA	Primary human fetal astrocytes
PI3K	Phosphotidylinositol-3-kinase
PKA	Protein kinase A
PKC	Protein kinase C
RISC	RNA-induced silencing complex
siRNA	Small interfering RNA
SND1	Staphylococcal nuclease domain containing 1
SNP	Single nucleotide polymorphism
TMD	Transmembrane domain
TNFα	Tumor necrosis factor α
VEGF	Vascular endothelial growth factor

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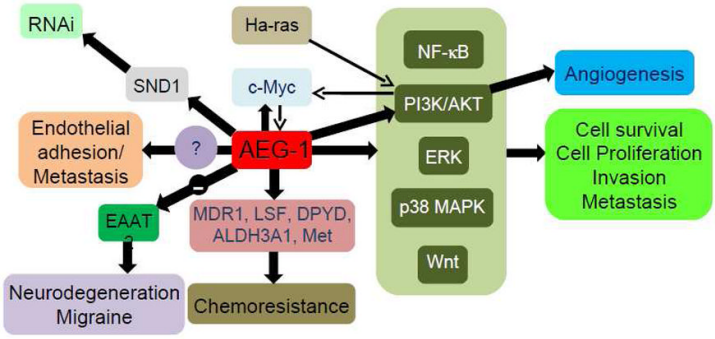

Fig. 1.

Molecular mechanism of AEG-1 action. Thick arrows indicate regulation by AEG-1 while thin arrows indicate mechanisms that regulate AEG-1. Arrow with "-" indicates negative regulation.

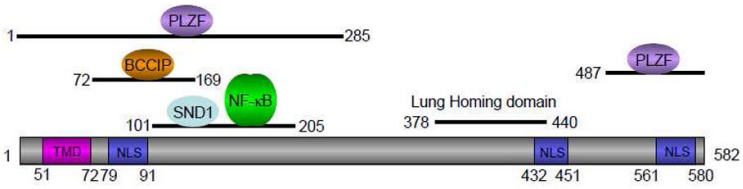

Fig. 2.

Schematic diagram of AEG-1 protein and the region that interacts with different proteins. The numbers represent amino acid positions. TMD: transmembrane domain; NLS: nuclear localization signal.

Table 1

Studies on AEG-1 as a diagnostic/prognostic biomarker in a variety of cancer types.

Cancer types	Cases of patients	References
HCC	109	Yoo et al., 2009
Breast cancer	225	Li et al., 2008
GBM	296	Liu et al., 2010
Brain cancer	98	Emdad et al., 2010
Prostate cancer	20	Kikuno et al., 2007
	143	Thirkettle et al., 2009
NSCLC	200	Song et al., 2009
ESSC	168	Yu et al., 2009
Neuroblastoma	10	Lee et al., 2009
Oligodendroglioma	75	Xia et al., 2010
Gastric cancer	105	Xu et al., 2010
Colorectal carcinoma	146	Song et al., 2010
Renal cell carcinoma	102	Chen et al., 2010