Published OnlineFirst November 10, 2009; DOI: 10.1158/0008-5472.CAN-09-1846

Review

# Astrocyte Elevated Gene-1: Far More Than Just a Gene Regulated in Astrocytes

Devanand Sarkar,[1,2,3] Luni Emdad,[4] Seok-Geun Lee,[1,3] Byoung Kwon Yoo,[1] Zao-zhong Su,[1] and Paul B. Fisher[1,2,3]

[1]Department of Human and Molecular Genetics, Virginia Commonwealth University; [2]Virginia Commonwealth University Institute of Molecular Medicine, Virginia Commonwealth University; [3]Virginia Commonwealth University Massey Cancer Center, Virginia Commonwealth University, School of Medicine, Richmond, Virginia; and [4]Department of Neurosurgery, Mount Sinai School of Medicine, New York, New York

## Abstract

**Since its original cloning by subtraction hybridization in 2002, it is now evident that Astrocyte elevated gene-1 (*AEG*-1) is a key contributor to the carcinogenic process in diverse organs. AEG-1 protein expression is elevated in advanced stages of many cancers, which correlates with poor survival. In specific cancers, such as breast and liver cancer, the *AEG*-1 gene itself is amplified, further supporting a seminal role in tumorigenesis. Overexpression and inhibition studies both in *in vitro* and in *in vivo* models reveal the importance of *AEG*-1 in regulating multiple physiologically and pathologically relevant processes including proliferation, invasion, metastasis, and gene expression. AEG-1 is a single-pass transmembrane protein with multiple nuclear localization signals and no known domains or motifs. Although pertinent roles of *AEG*-1 in the carcinogenic process are established, its potential function (promotion of metastasis only versus functioning as a bona fide oncogene) as well as localization (cell surface versus nucleus) remain areas requiring further clarification. The present review critically evaluates what is currently known about *AEG*-1 and provides new perspectives relative to this intriguing molecule that may provide a rational target for intervening in the cancer phenotype.** [Cancer Res 2009;69(22):8529–35]

## Introduction

With completion of the sequencing of the human genome, it is now evident that we only know the precise functions of very few genes, and there are a vast number of as-yet unexplored genes and their encoded proteins that might have profound roles in regulating key physiological and pathological events. This is particularly relevant for cancer in which novel genetic and epigenetic changes are being discovered every day. New candidate genes from previously unappreciated pathways, such as ubiquitination, metabolism, and mRNA biogenesis, are being identified, which regulate the processes of tumorigenesis and cancer progression. This accrued knowledge is allowing researchers to develop strategies targeting novel molecules and unique aspects of carcinogenesis, thereby ushering in optimism that prolonged survival in cancer patients will be an achievable objective. Astrocyte elevated gene-1 (*AEG*-1) represents an important genetic determinant regulating multiple events in tumorigenesis. Following its initial cloning in 2002 (1), *AEG*-1 has become the center of attention in an increasing spectrum of tumor indications for its multiple roles in regulating cancer progression and metastasis. The present review provides a current perspective of our understanding of this very interesting gene.

## Cloning of *AEG*-1 as a Novel Gene Modulated in Normal Human Fetal Astrocyte Treated with Neurotoxic Agents

*AEG*-1 was first cloned in 2002 as a human immunodeficiency virus (HIV)-1- and tumor necrosis factor (TNF)-α-inducible gene in primary human fetal astrocytes (PHFA; ref. 1). HIV-1 causes a low productive, noncytolytic infection of astrocytes and does not infect neurons (2, 3). However, late stage acquired immunodeficiency syndrome (AIDS) is associated with neuronal degeneration and dementia (HIV-associated dementia or HAD), and it is hypothesized that changes in astrocyte function following HIV infection is a primary contributor to neuronal death (2). In an effort to elucidate gene expression changes in HIV-1-infected astrocytes, a rapid subtraction hybridization (RaSH) approach was done between HIV-1-infected and -noninfected PHFA (1). A series of HIV-1-induced (termed astrocyte elevated genes or AEGs) and HIV-1-suppressed (termed astrocyte suppressed genes or ASGs) genes were identified by this method (1). *AEG*-1 was shown to be induced by HIV-1, its cell surface protein gp120, or by HIV-1-induced cytokine tumor necrosis factor-α (TNF-α) at 3 to 7 days following treatment, indicating that *AEG*-1 induction by HIV-1 is a delayed and sustained event (1, 4). As yet, the role of *AEG*-1 induction in the alteration of astrocyte function or generation of HAD has not been elucidated. However, expression analysis revealed that *AEG*-1 expression is significantly elevated in cancer cells when compared with normal cells, thereby highlighting novel and unanticipated functions of *AEG*-1 in tumorigenesis (discussed below).

In a separate approach in 2004, *in vivo* phage screening resulted in the cloning of mouse *AEG*-1 as a protein mediating metastasis of mouse breast cancer cells to lung and was named metadherin (5). A phage library, enriched for secreted and transmembrane proteins from mouse 4T1 breast cancer cells, was injected intravenously in mouse, and phage that localized to the lungs were isolated and sequenced. One of the clones encoded the amino acid 378-440 of mouse *AEG*-1, which was designated as the "lung homing domain" of the protein. In the same year, the mouse-rat *AEG*-1 was also cloned as a tight junction protein named LYRIC and also

**Requests for reprints:** Paul B. Fisher, Virginia Commonwealth University, School of Medicine, 1101 East Marshall Street, Sanger Hall Building, Room 11-015, Richmond, VA 23298. Phone: 804-828-9632; Fax: 804-827-1124; E-mail: pbfisher@vcu.edu and Devanand Sarkar, Virginia Commonwealth University, School of Medicine, 1220 E. Broad St., P.O. Box 980035, Richmond, VA 23298. Phone: 804-827-2339; Fax: 804-628-1176; E-mail: dsarkar@vcu.edu.



doi:10.1158/0008-5472.CAN-09-1846

Published OnlineFirst November 10, 2009; DOI: 10.1158/0008-5472.CAN-09-1846



by gene trapping techniques and was named 3D3/lyric (6, 7). Human *AEG*-1 mRNA encodes a single-pass transmembrane protein of predicted molecular mass of ~64 kDa and pI of 9.3 (4). Human *AEG*-1 gene consists of 12 exons and 11 introns and is located at chromosome 8q22 (4). This location is especially significant because amplification of 8q22 has been shown in a number of malignancies such as malignant glioma, hepatocellular carcinoma (HCC), and breast cancer, and indeed genomic amplification of *AEG*-1 has been identified in HCC and breast cancer patients (8–10). Human AEG-1 is a lysine-rich highly basic protein consisting of 582 amino acid residues (4, 11). It has three putative nuclear localization signals, between 79-91 amino acid residues, 432-451 amino acid residues, and 561-580 amino acid residues (12). There is a putative transmembrane domain (TMD) between 51-72 amino acid residues. A lung homing domain has been identified in mouse *AEG*-1 (metadherin) that corresponds to 381-443 amino acid residues of human *AEG*-1 (5). Additionally, the molecule also contains an *N*-terminal "LXXLL" motif that is employed by transcriptional co-activators to interact with transcription factors (13).

**Expression analysis of AEG-1 reveals a direct link with tumorigenesis in multiple organs.** Multitissue Northern blots containing total RNA of normal human organs revealed that *AEG*-1 mRNA is ubiquitously expressed at varying levels in all organs (4). Expression analysis in cell lines revealed that *AEG*-1 expression is significantly higher in breast, prostate, esophageal, and liver cancer, and melanoma, malignant glioma, and neuroblastoma cell lines in comparison to their normal counterparts (4, 9, 10, 14–16). These observations in cell lines have been confirmed in patient-derived tumor samples mainly by immunohistochemistry in tissue microarrays (TMA). In TMA containing 9 normal liver samples and 109 human HCC samples of different stages and grades, all 9 normal liver samples showed very low to undetectable expression of AEG-1 (9). Seven (7) out of the 109 human HCC samples showed no staining for AEG-1. Out of the 102 AEG-1-positive human HCC samples (93.58%), a statistically significant correlation between AEG-1 expression level and disease stage were obtained (9). Additionally, mining of existing Affymetrix gene expression data from 132 HCC patients revealed a statistically significant increase in *AEG*-1 mRNA expression in HCC patients compared with normal liver or cirrhotic patients (9). Strong AEG-1 staining was observed in 17 out of 31 samples of breast carcinoma patients, whereas AEG-1 staining was absent in 18 out of 20 samples of normal breast tissue (5). A study from China analyzing 225 breast cancer patients showed increased expression of the protein in 44.5% of cases that correlated with the progression of the disease (17). Moreover, AEG-1 expression inversely correlated with patient survival. Interestingly, in this study, the metastatic tumors showed more nuclear staining of AEG-1 (17). In a separate study analyzing 170 breast cancer patients, 47% showed moderate to high level of AEG-1 expression, and AEG-1 expression was significantly associated with a higher risk of metastasis indicating that AEG-1 might be a prognostic factor for this disease (10). However, in tissue sections of human breast cancer used in this study, AEG-1 protein was localized predominantly in the cytoplasm. A study using 20 benign prostatic hyperplasia (BPH) and 20 prostate cancer patient samples revealed strong positive AEG-1 expression in 80% of the prostate cancer patients compared with only 10% of the BPH cases (14). In a separate study using TMA (tissue microarray) of 143 prostate cancer and 63 BPH cases, increased AEG-1 staining was observed in the cancer patients compared with BPH patients (12). Interestingly, in this study more nuclear staining of AEG-1 was observed in BPH patients compared with prostate cancer patients, and decreased nuclear staining of AEG-1 was associated with increased Gleason grade and lower survival rate (12). The authors hypothesized that nuclear AEG-1 might have a function in normal prostate epithelial cells that is lost during tumorigenesis. In a separate group of 11 patients with prostate cancer bone metastasis, 81.8% of prostate bone metastasis showed higher AEG-1 expression compared with normal bone (12). Analysis of 168 esophageal squamous cell carcinoma (ESCC) patients revealed high AEG-1 expression in 92.9% cases compared with normal esophageal tissue, and AEG-1 expression inversely correlated with patient survival (15). Expression analysis using 2 normal peripheral nerve tissue and 10 neuroblastoma tissues revealed high AEG-1 staining in neuroblastoma, whereas no staining was observed in the peripheral nerves (16). Our recent studies with 7 normal skin, 22 nevi, 15 radial growth phase (RGP) melanoma, 25 vertical growth phase (VGP) melanoma, and 12 lymph node metastasis of melanoma revealed strong AEG-1 expression in VGP and metastasis when compared with the benign disease or RGP melanoma.[5] Interestingly, in addition to cytoplasmic staining, nuclear AEG-1 staining was observed in VGP and metastatic melanoma but not in normal melanocytes, nevi, or RGP melanoma. Our recent findings in malignant glioma patients also reveal high AEG-1 expression in >90% of cases compared with the normal brains.[6]

**Phenotypes resulting from AEG-1 modulation support its seminal role in oncogenesis.** Gain-of-function (overexpression) and loss-of-function (inhibition) studies are providing insights into the functional significance of *AEG*-1 in the process of tumorigenesis. Experimental approaches to define functions included proliferation (analyzed by MTT, colony formation assay, and soft agar assay), migration, matrigel invasion, and *in vivo* tumorigenesis and metastasis assays. Overexpression of *AEG*-1 resulted in increased proliferation of human liver and esophageal cancer, malignant glioma, and neuroblastoma and melanoma cells (9, 15, 16).[5] Enhanced expression of *AEG*-1 also increased matrigel invasive potential of HeLa, human HCC, neuroblastoma, malignant glioma and melanoma cells, and cloned rat embryonic fibroblasts (CREF; refs. 9, 13, 16, 18, 19). As a corollary, knockdown of *AEG*-1 inhibited proliferation of human prostate cancer, neuroblastoma and melanoma cells, and induced apoptosis in prostate cancer and neuroblastoma cells (14, 16, 20). *AEG*-1 knockdown also inhibited invasion of human prostate cancer, neuroblastoma, malignant glioma, and melanoma cell lines (14, 20).[5,6] Interestingly, neither overexpression nor inhibition altered any of these *in vitro* properties of human breast cancer cells (10), although a recent study documents that overexpression of *AEG*-1 does increase proliferation and invasion of human breast cancer cells and knockdown of *AEG*-1 reverses these effects (21). The reasons for these discrepant findings remain to be determined. Overexpression of *AEG*-1 in normal human cells, such as primary normal human fetal astrocytes (PHFA), normal immortal melanocytes (FM516-SV), and normal cloned rat embryonic fibroblasts (CREF), protects them from serum starvation-induced apoptosis indicating that as an anti-apoptotic protein *AEG*-1 might function as an oncogene (22). AEG-1 can synergize with Ha-*ras* to augment the transformed phenotype in immortal

[5] H. Boukerche, B.K. Yoo, L. Emdad, et al. Role of Astrocyte Elevated Gene-1 (AEG-1) in regulating melanoma progression, manuscript in preparation.

[6] L. Emdad, D. Sarkar, S.-G. Lee, et al. Astrocyte Elevated Gene-1 (AEG-1): a novel target for human glioma therapy, submitted for publication.

Published OnlineFirst November 10, 2009; DOI: 10.1158/0008-5472.CAN-09-1846



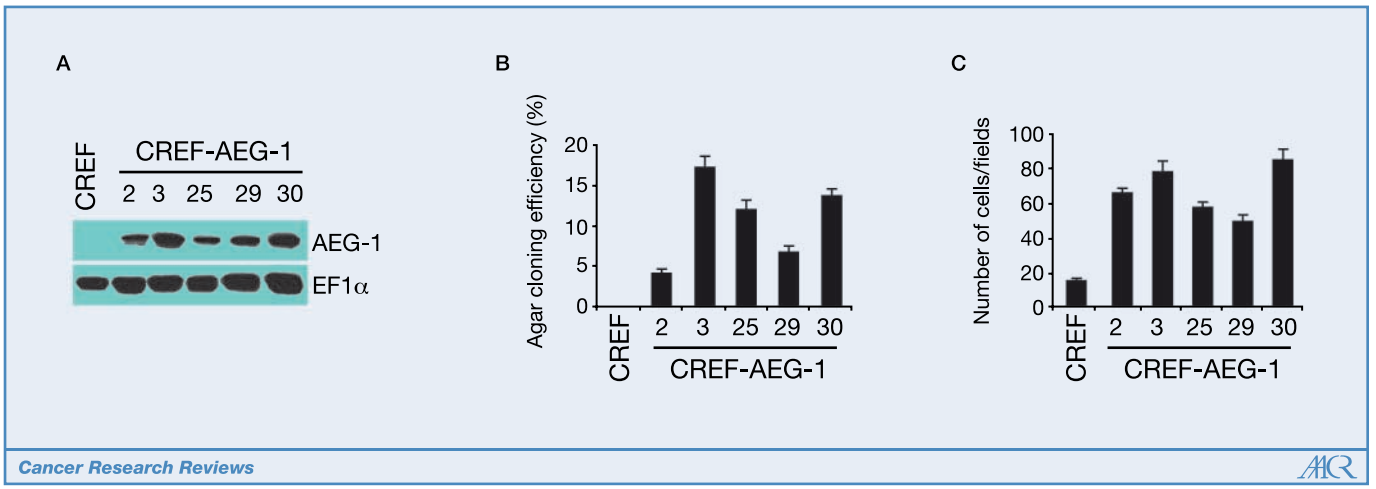


**Figure 1.** Effects of stable overexpression of AEG-1 in normal immortal CREF cells on colony formation in soft agar and cell invasion. *A*, CREF cells were stably transfected with either the empty pcDNA3.1 vector or an AEG-1 expression vector, respectively. CREF-AEG-1 clones were selected for expression of AEG-1. Expression of AEG-1 protein by stably transfected CREF-AEG-1 cells is shown by Western blot analysis. EF1α was used as an internal control to confirm equal loading. *B*, a total of $1 \times 10^5$ cells were seeded in 0.4% agar on 0.8% base agar. Two weeks later, colonies >0.1 mm were counted under a dissection microscope. *C*, quantitation of the invasion assay. Cells ($5 \times 10^4$) were seeded onto the upper chamber of a matrigel invasion chamber system in the absence of serum. Twenty-four hours after seeding, the filters were fixed, stained, and photographed. The data expressed in the graph are the mean ± standard error of three independent experiments.

SV40 T-antigen-expressing human melanocytes (FM516-SV), as well as in PHFA, further supporting an oncogenic function of *AEG*-1 (4). With the exception of immortality, CREF do not exhibit transformation-associated properties (19). However, stable overexpression of *AEG*-1 in CREF results in morphological transformation with increased invasion and soft agar growth showing that *AEG*-1 alone can function as an oncogene for rodent cells (Fig. 1; ref. 19).

In *in vivo* assays, using nude mice xenograft models, overexpression of *AEG*-1 in human HCC cells resulted in highly aggressive, angiogenic and metastatic tumors, whereas inhibition of *AEG*-1 abrogated subcutaneous tumor formation by human HCC, neuroblastoma, and melanoma cells and intracerebral tumor formation by human glioma cells (refs. 9, 16; Fig. 2).[5,6] Similarly, CREF-*AEG*-1 cells also generated highly aggressive and angiogenic tumors in nude mice (19). The tumor sections revealed augmented expression of specific angiogenesis molecules including angiopoietin-1 (Ang1), matrix metalloprotease (MMP)-2, and HIF1-α (19). Supporting the angiogenic functions of *AEG*-1, overexpression of *AEG*-1 augmented tube formation of human umbilical vein endothelial cells (HUVEC), whereas siRNA inhibition of *AEG*-1 blocked vascular endothelial growth factor (VEGF)-induced tube formation of HUVEC in matrigel (19). In an *in vivo* chicken chorioallantoic membrane (CAM) assay, *AEG*-1 siRNA inhibited capillary neovascularization of H4 human glioma cells confirming the pro-angiogenic properties of *AEG*-1 (19). Overexpression of *AEG*-1 augmented *in vivo* metastasis of human breast cancer cells and HEK293T cells especially to the lungs, whereas inhibition of *AEG*-1 reversed this phenomenon for both human and mouse breast cancer cells (5, 10). Animals injected with *AEG*-1-overexpressing breast cancer cells had decreased survival rate, whereas *AEG*-1 inhibition increased the survival rate (10). It has been shown that overexpression of *AEG*-1 increased adhesion of breast cancer cells to the endothelium, whereas inhibition of *AEG*-1 abrogated this phenomenon (10). This increased endothelial adhesion has been postulated to be the mechanism of increased metastasis by *AEG*-1. However, the AEG-1-interacting molecule on the endothelial cells has not been identified.

**Molecular mechanism of AEG-1 action, new insights into the functions of this intriguing molecule.** AEG-1 promotes tumorigenesis by modulating multiple signal transduction pathways and altering global gene expression changes (Fig. 3 and Table 1). The first signaling pathway identified as being activated by *AEG*-1 was nuclear factor κ-B (NF-κB; ref. 18). In HeLa cells and human malignant glioma cells, upon TNF-α treatment (which induces *AEG*-1 expression), AEG-1 translocates into the nucleus where it interacts with the p65 subunit of NF-κB and augments NF-κB-induced gene expression (13, 18). Although AEG-1 does not directly bind to DNA, upon TNF-α treatment it interacts with p65 and CBP on the IL-8 promoter increasing IL-8 transcription (13). IL-8, an NF-κB downstream gene, positively regulated angiogenesis and metastasis, and inhibition of NF-κB abrogated AEG-1-induced augmentation of soft agar growth and matrigel invasion by HeLa cells (18). Deletion mutant analysis showed that the *N*-terminal 71 amino acids that contain the transmembrane domain and the LXXLL motif are important in mediating AEG-1-induced invasion, soft agar growth, and NF-κB activation (13). However, the p65-interaction domain of AEG-1 was mapped to 101-205 amino acids of AEG-1 (13). The LXXLL motif of AEG-1 might mediate its interaction with CBP, which provides a crucial connection of the AEG-1-NF-κB complex to the basal transcriptional machinery. This hypothesis needs to be experimentally proven by site-directed mutagenesis studies. NF-κB activation by AEG-1 has also been recently documented in prostate and liver cancer cells (9, 14). Interestingly, in human promonocytic cells, LPS treatment resulted in induction of AEG-1 expression by NF-κB activation (23). On the other hand, AEG-1 itself activated NF-κB, and inhibition of AEG-1 prevented LPS-induced production of proinflammatory cytokines, such as TNF-α and PGE2 (23). These findings suggest a potential immunological role of AEG-1 in bacterial infection, as well as a key role of AEG-1 in inflammatory mechanisms of cancer.

Published OnlineFirst November 10, 2009; DOI: 10.1158/0008-5472.CAN-09-1846



A second pathway modulated by AEG-1 is the PI3K/Akt pathway. Interestingly, this pathway is not only activated by AEG-1 but also plays a key role in regulating AEG-1 expression (24). AEG-1 expression is significantly augmented by Ha-*ras*. Ha-*ras* activates the PI3K/Akt pathway that leads to binding of the transcription factor c-Myc to the E-box element in the promoter region of AEG-1 and induces AEG-1 transcription (24). AEG-1 in turn activates the PI3K/Akt pathway, by a mechanism not yet elucidated, which plays an important role in providing protection from serum starvation-induced apoptosis of normal cells (22). Whereas c-Myc induces AEG-1 transcription, AEG-1 also induces c-Myc expression, and in neuroblastoma cells it induces *N*-Myc expression, thereby amplifying the tumorigenic effect (16, 24). By activating Akt, AEG-1 down-regulates pro-apoptotic Bad and p21 and upregulates MDM2, nullifying p53 function, thus exerting its anti-apoptotic effect (24). Inhibition of AEG-1 in prostate cancer cells down-regulates Akt activation and leads to upregulation of forkhead box (FOXO)3a activity and p27, resulting in apoptosis (14). In esophageal cancer cells, activation of Akt by AEG-1 leads to upregulation of cyclin D1 and down-regulation of p27 (15). The PI3/Akt signaling pathway has also been shown to regulate *AEG*-1-induced angiogenesis (19). Dominant negative inhibition of Akt abrogated *AEG*-1-induced tube formation by HUVECs, inhibited *AEG*-1-induced expression of angiogenic markers Tie-2 and HIF-1α in HUVECs and U87 human glioma cells, and inhibited *AEG*-1-induced activation of the VEGF promoter (19).

In HCC cells, in addition to the NF-κB and Akt pathways, AEG-1 also activates the MAP kinase pathway, notably the MEK/ERK and p38 MAPK pathways, and inhibition of either of these pathways result in abrogation of AEG-1-induced invasion of HCC cells (9). AP-1, an oncogenic transcription factor downstream of MEK/ERK, has been shown to be activated by AEG-1 in human prostate cancer cells (14).

Microarray analysis focused on identifying AEG-1 downstream genes resulted in identification of several important pathways and molecules contributing to carcinogenesis. In HCC cells, AEG-1 overexpression resulted in alteration of a plethora of genes involved in invasion, senescence, chemoresistance, angiogenesis, and metastasis, all inexorably linked to HCC pathogenesis (9). AEG-1 activates Wnt/β-catenin signaling via ERK42/44 activation, leading to β-catenin nuclear translocation, and upregulates LEF-1/TCF-1, the ultimate executor of the Wnt pathway (9). Inhibition studies show that activation of Wnt signaling plays a key role in mediating AEG-1 function. In breast cancer cells, similar microarray approaches identified down-regulation of two cell death-inducing genes TRAIL and BINP3 by *AEG*-1 (10).

One important attribute of AEG-1 is its ability to confer resistance to chemotherapeutic agents in human HCC, breast cancer, and neuroblastoma cell lines (9, 10, 20). Microarray analysis identified several key genes contributing to chemoresistance in HCC cells (9). *AEG*-1 induces the transcription factor LSF (Late SV40 Factor) that directly upregulates thymidylate synthase (TS; ref. 25). 5-fluorouracil (5-FU) is converted intracellularly into its active metabolite FdUMP, which inhibits TS, thus reducing the thymidine pool and increasing the uracil pool leading to the inhibition of DNA synthesis. Additionally, *AEG*-1 induces the 5-FU catabolizing enzyme dihydropyrimidine dehydrogenase (DPYD). The

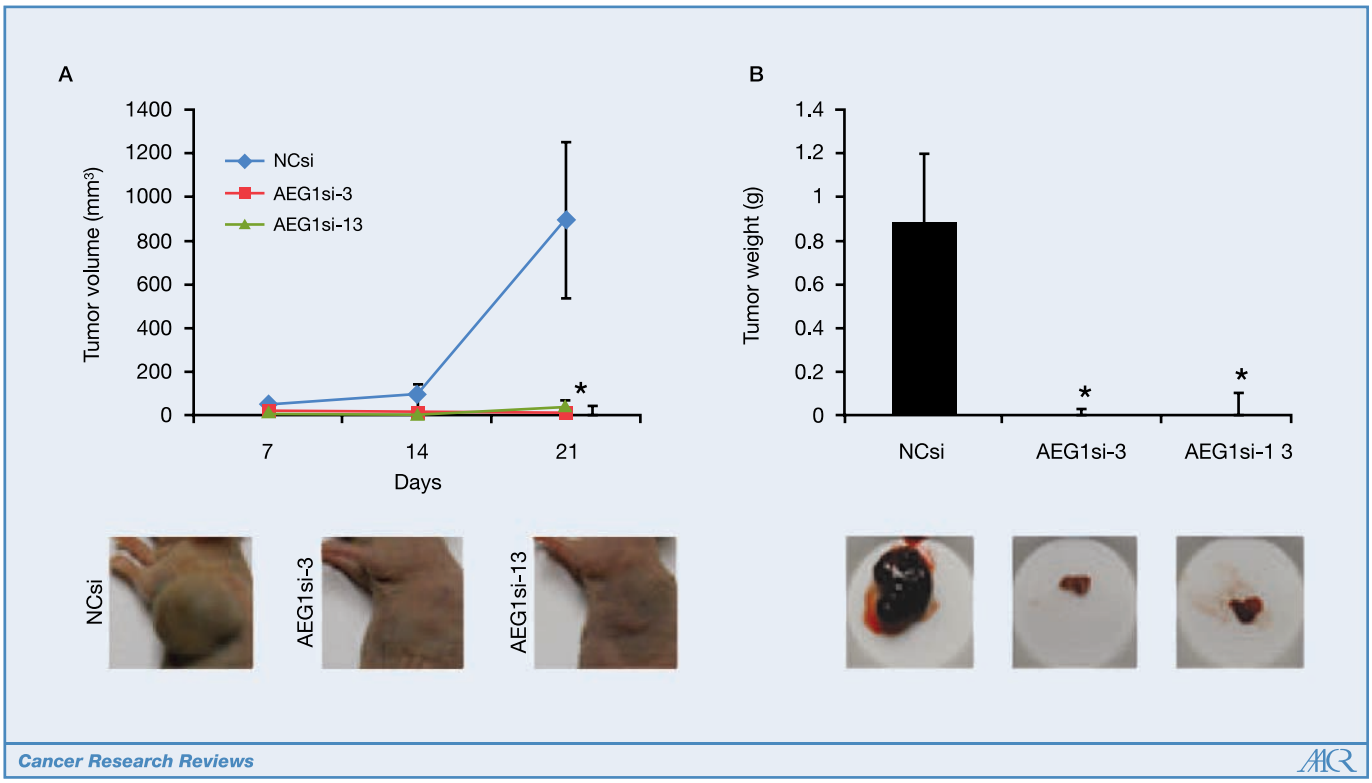


**Figure 2.** Knockdown of AEG-1 blocks tumorigenesis of BE(2)-C human neuroblastoma cells *in vivo*. NCsi (control siRNA), AEG-1si-3, and AEG-1si-13 clones were established in BE(2)-C cells. Cells ($1 \times 10^6$) were subcutaneously implanted in the flanks of athymic nude mice and tumor volume *A,* and tumor weight *B,* were measured 21 days later. The data represent mean ± standard error of the mean with five animals in each group ($P < 0.05$ versus NCsi).

Published OnlineFirst November 10, 2009; DOI: 10.1158/0008-5472.CAN-09-1846



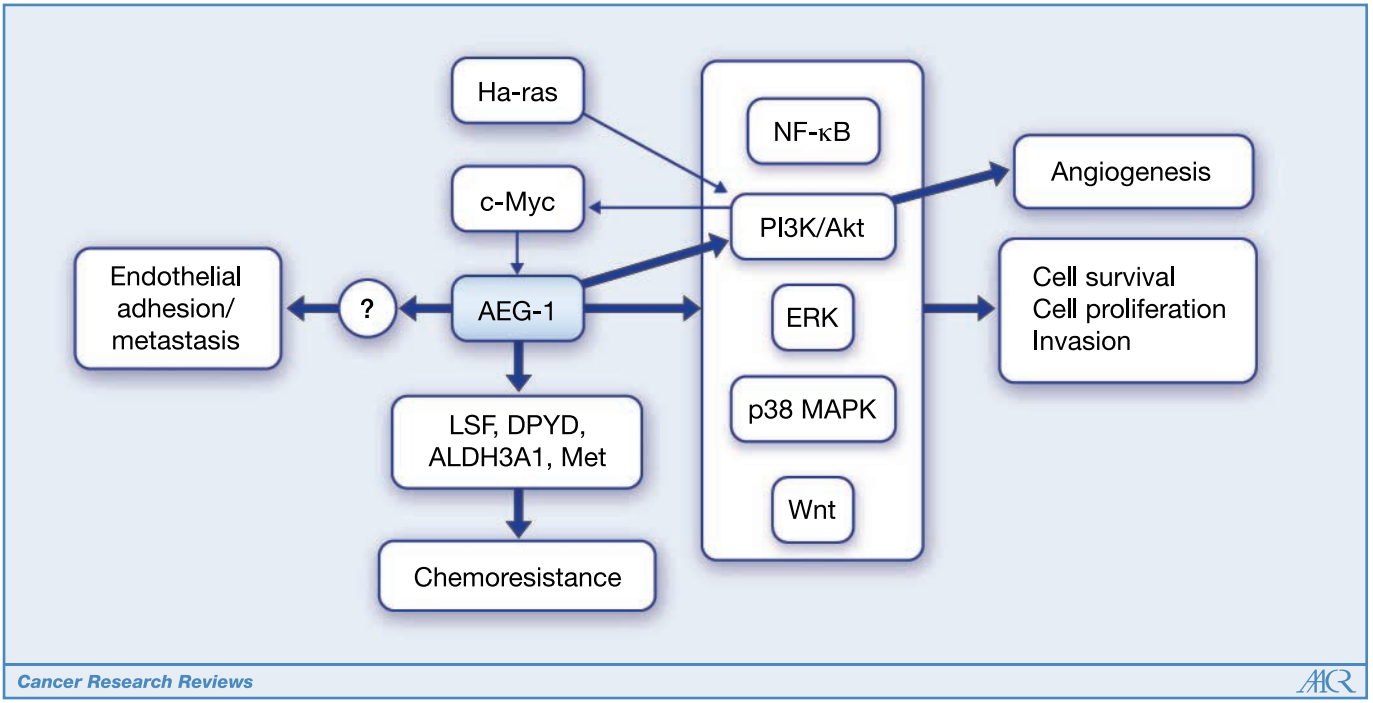


**Figure 3.** A hypothetical model of the signal transduction pathways regulating AEG-1 expression and AEG-1-mediated tumorigenesis. Thick arrows denote signaling pathways and/or molecules modulated by AEG-1, whereas thin arrows denote signaling pathways and/or molecules regulating AEG-1 expression. See text for details.

combined induction of LSF and DPYD by AEG-1 contributes to 5-FU resistance in HCC cells (25). In breast cancer cells, *AEG*-1 induces several genes related to chemoresistance, of which the aldehyde dehydrogenase 3 family, member A1 (ALDH3A1) and the hepatocyte growth factor receptor (Met) were shown to play a role in mediating resistance to doxorubicin, paclitaxel, and 4-hydroxycyclophosphamide (4-HC; ref. 10).

Recent studies have identified BCCIPα [BRCA-2 and CDKN1A (p21)-associated protein] as a potential interacting partner of AEG-1 (26). BCCIPα binds to p21 and enhances p21-mediated inhibition of Cdk2 kinase. Loss of BCCIP impairs $G_1$/S checkpoint activation following DNA damage and in conjunction with BRCA2, BCCIP plays a role in homologous recombination repair of DNA damage and contributes to maintenance of chromosome stability. BCCIPα expression is down-regulated in breast cancer and glioma cells and overexpression of BCCIPα inhibits cell growth. A yeast-two hybrid screen identified BCCIPα as a potential-interacting partner with AEG-1, and the *N*-terminal 169 amino acid residues of AEG-1 were shown to mediate this interaction (26). AEG-1 overexpression results in enhanced proteasomal degradation of BCCIPα. However, whether interaction of AEG-1 with BCCIPα followed by BCCIPα down-regulation plays any role in AEG-1-mediated invasion or tumor progression was not studied.

**Localization and post-translational modification of AEG-1.** There are some disputed issues about the potential localization of AEG-1 and its function. As mentioned earlier, AEG-1 was first cloned in 2002 as an HIV-1- and TNF-α-induced gene and subsequently cloned and reported by multiple groups in 2004. It was cloned as LYRIC by Britt and colleagues as a protein localized to tight junctions in polarized rat and human prostate epithelial cells, and by immunofluorescence LYRIC showed co-localization with the tight junction protein ZO-1 (6). In the same study it was shown that although in normal rat hepatocytes, LYRIC localized in the tight junctions, in rat hepatoma cells AS30D as well as in human 293T cells overexpressing LYRIC, the protein was localized in the perinuclear region (6). The perinuclear localization of the protein, cloned as 3d3/lyric, was also shown by Sutherland and colleagues (7). 3d3/lyric was detected in the endoplasmic reticulum (ER) and perinuclear region as well as in nucleus, especially nucleolus, in HeLa cells and human fibrosarcoma cells HT1080 (7). On the other hand, Brown and Ruoslahti identified metadherin as a cell surface protein in mouse breast cancer cells and HEK293T cells overexpressing metadherin (5). It was hypothesized that the large C-terminal domain of the protein is displayed outside of the cell and facilitates homing of breast cancer cells to the lung and promotes metastasis (5). In human HCC cells, in an unpermeabilized condition, AEG-1 protein is detected only on the cell surface, whereas upon permeabilization, it is detected both in the cytoplasm as well as in the nucleus.[7] We also observed nuclear staining in human HCC samples.[7] In HeLa cells and malignant glioma cells, TNF-α treatment results in nuclear translocation of AEG-1 (13, 18). A recent study narrowed down the nuclear localization signals (NLS) of AEG-1 by deletion and mutation studies (12). It was shown that extended sequences outside of the canonical NLS (exNLS) are important to determine AEG-1 localization. The exNLS-3 (546-582 amino acids) predominantly mediates nuclear localization, whereas ex-NLS-1 (78-130 amino acids) regulates nucleolar localization (12). Although the putative molecular mass of AEG-1 is ~64 kDa, in Western blot analysis the predominant band migrates at ~75

[7] B.K. Yoo and D. Sarkar, unpublished data.

Published OnlineFirst November 10, 2009; DOI: 10.1158/0008-5472.CAN-09-1846



**Table 1.** Signaling pathways and/or molecules modulated by AEG-1 to induce specific phenotypes in different cell types

| Cell Type | Signaling Pathways and/or Molecules | Phenotype | Reference |
|---|---|---|---|
| Malignant glioma; Cervical, prostate, esophageal and hepatocellular carcinoma | NF-κB | Migration and invasion | 9, 13, 15, 18 |
| Promonocytic cells | NF-κB | Production of pro-inflammatory cytokines | 23 |
| Malignant glioma; Prostate, esophageal and hepatocellular carcinoma; Neuroblastoma; Immortal normal cells, e.g., melanocytes, astrocytes, rodent fibroblasts | PI3K/Akt | Cell survival and anti-apoptosis | 9, 14–16, 19, 22 |
| Human vascular endothelial cells | PI3K/Akt | Angiogenesis | 19 |
| Malignant glioma; HCC | c-Myc | Cell survival and Invasion | 9, 22 |
| Neuroblastoma | N-Myc | Cell survival and Invasion | 16 |
| HCC | MEK/ERK and p38 MAPK | Cell survival and Invasion | 9 |
| HCC | Wnt/β-catenin | Invasion | 9 |
| HCC | LSF, DPYD | Resistance to 5-FU | 25 |
| Breast cancer | ALDH3A1, Met | Chemoresistance | 10 |
| Breast cancer | ? | Endothelial adhesion and metastasis | 5, 10 |

NOTE: Please see text for more details.

kDa followed by an ~64-kDa band. It was shown that exNLS-2 (415-486 amino acids) of AEG-1 is mono-ubiquitinated resulting in the ~75-kDa band that retains AEG-1 in the cytoplasm (12). However, the functional properties of the generated constructs were not analyzed. Does a mutant that cannot localize to the nucleus or nucleolus have comparable functional activity as wild-type AEG-1? Similarly, what is the biological behavior of a mutant that cannot be ubiquitinated?

## Conclusion and Future Perspectives

Based on *in vitro* and *in vivo* studies in animals and expression analysis using patient samples, it is eminently clear that *AEG*-1 plays a decisive role in the process of tumorigenesis in multiple organs. However, despite our expanded understanding of this interesting gene, a large number of unanswered questions and areas requiring clarification need to be addressed. One important question is whether *AEG*-1 only promotes metastasis, as suggested by two studies in breast cancer, or does it also regulate crucial events earlier in the carcinogenic process, such as immortalization, transformation, and increased proliferation as suggested by multiple studies in other cancer indications as well as using immortal normal cells? Simply put, can *AEG*-1 function as a *bona fide* oncogene or does *AEG*-1 require an initial transforming event to allow tumor progression? The answer to this question might be obtained by analyzing whether AEG-1 overexpression alone might immortalize or transform primary normal cells, and from AEG-1-overexpressing transgenic mouse models. The relationship between localization of AEG-1 and its function is also another area that requires resolution. In prostate cancer, decreased nuclear staining of AEG-1 correlates with poor prognosis, whereas in breast cancer, HCC, and melanoma advanced disease is associated with increased AEG-1 nuclear staining. What is the role of nuclear AEG-1 in regulating the tumorigenic process? Apart from NF-κB, does *AEG*-1 function as a transcriptional co-activator for any other transcription factor (s)? What is the receptor for cell surface AEG-1 that allows it to home to the endothelium? Can cell surface AEG-1 be targeted by a radionucleotide-coupled antibody approach for diagnosis and treatment of metastatic disease? Is it possible to develop a small molecule inhibitor of AEG-1? Elucidation of the crystal structure of AEG-1 will facilitate identification of a small molecule inhibitor.

AEG-1 is a distinctive molecule that has no homolog and it has no known domains or motifs. Thus, identification of AEG-1-interacting proteins might provide clues about its mechanism of action. Although AEG-1 protein expression is increased in a significant percentage of patients, the gene itself is amplified in a small subset of patients, and the relevance of this amplification to phenotype requires further investigation. This raises the obvious question about what other mechanisms elevate AEG-1 expression in cancer cells?

As emphasized in this mini-review, AEG-1 is an intriguing molecule whose multiple functions continue to be elucidated. Over time and with increased investigation by multiple laboratories, many of the questions we raise will be answered and a more precise understanding of the role of this gene product in normal and abnormal physiology will be forthcoming. Expanded research will also validate the potential effectiveness of AEG-1 as a target for intervening in cancer progression and metastasis.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

## Acknowledgments

Received 5/22/09; revised 7/22/09; accepted 7/25/09; published OnlineFirst 11/10/09.

**Grant support:** National Institutes of Health grants P01 CA104177, R01 CA097318, R01 CA134721, and P01 NS31492; the National Foundation for Cancer Research (NFCR); and the Samuel Waxman Cancer Research Foundation (SWCRF) to P.B. Fisher and awards from the Goldhirsh Foundation for Brain Cancer Research and the Dana Foundation to D. Sarkar. D. Sarkar is the Harrison Endowed Scholar in the VCU Massey Cancer Center. P.B. Fisher holds the Thelma Newmeyer Corman Endowed Chair in Cancer Research in the VCU Massey Cancer Center and is an SWCRF Investigator.

Published OnlineFirst November 10, 2009; DOI: 10.1158/0008-5472.CAN-09-1846



## References


1. Su ZZ, Kang DC, Chen Y, et al. Identification and cloning of human astrocyte genes displaying elevated expression after infection with HIV-1 or exposure to HIV-1 envelope glycoprotein by rapid subtraction hybridization, RaSH. Oncogene 2002;21:3592–602.
2. Brack-Werner R. Astrocytes: HIV cellular reservoirs and important participants in neuropathogenesis. AIDS 1999;13:1–22.
3. Lipton SA, Gendelman HE. Seminars in medicine of the Beth Israel Hospital, Boston. Dementia associated with the acquired immunodeficiency syndrome. N Engl J Med 1995;332:934–40.
4. Kang DC, Su ZZ, Sarkar D, Emdad L, Volsky DJ, Fisher PB. Cloning and characterization of HIV-1-inducible astrocyte elevated gene-1, AEG-1. Gene 2005;353:8–15.
5. Brown DM, Ruoslahti E. Metadherin, a cell surface protein in breast tumors that mediates lung metastasis. Cancer Cell 2004;5:365–74.
6. Britt DE, Yang DF, Yang DQ, et al. Identification of a novel protein, LYRIC, localized to tight junctions of polarized epithelial cells. Exp Cell Res 2004;300:134–48.
7. Sutherland HG, Lam YW, Briers S, Lamond AI, Bickmore WA. 3D3/lyric: a novel transmembrane protein of the endoplasmic reticulum and nuclear envelope, which is also present in the nucleolus. Exp Cell Res 2004;294:94–105.
8. Warr T, Ward S, Burrows J, et al. Identification of extensive genomic loss and gain by comparative genomic hybridisation in malignant astrocytoma in children and young adults. Genes Chromosomes Cancer 2001;31:15–22.
9. Yoo BK, Emdad L, Su ZZ, et al. Astrocyte elevated gene-1 regulates hepatocellular carcinoma development and progression. J Clin Invest 2009;119:465–77.
10. Hu G, Chong RA, Yang Q, et al. MTDH activation by 8q22 genomic gain promotes chemoresistance and metastasis of poor-prognosis breast cancer. Cancer Cell 2009;15:9–20.
11. Emdad L, Sarkar D, Su ZZ, et al. Astrocyte elevated gene-1: recent insights into a novel gene involved in tumor progression, metastasis and neurodegeneration. Pharmacol Ther 2007;114:155–70.
12. Thirkettle HJ, Girling J, Warren AY, et al. LYRIC/AEG-1 is targeted to different subcellular compartments by ubiquitinylation and intrinsic nuclear localization signals. Clin Cancer Res 2009;15:3003–13.
13. Sarkar D, Park ES, Emdad L, Lee SG, Su ZZ, Fisher PB. Molecular basis of nuclear factor-κB activation by astrocyte elevated gene-1. Cancer Res 2008;68:1478–84.
14. Kikuno N, Shiina H, Urakami S, et al. Knockdown of astrocyte-elevated gene-1 inhibits prostate cancer progression through upregulation of FOXO3a activity. Oncogene 2007;26:7647–55.
15. Yu C, Chen K, Zheng H, et al. Overexpression of astrocyte elevated gene-1 (AEG-1) is associated with esophageal squamous cell carcinoma (ESCC) progression and pathogenesis. Carcinogenesis 2009;30:894–901.
16. Lee SG, Jeon H-Y, Su ZZ, et al. Astrocyte Elevated Gene-1 contributes to the pathogenesis of neuroblastoma. Oncogene 2009;28:2476–84.
17. Li J, Zhang N, Song LB, et al. Astrocyte elevated gene-1 is a novel prognostic marker for breast cancer progression and overall patient survival. Clin Cancer Res 2008;14:3319–26.
18. Emdad L, Sarkar D, Su ZZ, et al. Activation of the nuclear factor κB pathway by astrocyte elevated gene-1: implications for tumor progression and metastasis. Cancer Res 2006;66:1509–16.
19. Emdad L, Lee SG, Su Z-Z, Boukerche H, Sarkar D, Fisher PB. Astrocyte elevated gene-1 (AEG-1) functions as an oncogene and regulates angiogenesis. Proc Natl Acad Sci U S A. In press 2009.
20. Liu H, Song X, Liu C, Xie L, Wei L, Sun R. Knockdown of astrocyte elevated gene-1 inhibits proliferation and enhancing chemo-sensitivity to cisplatin or doxorubicin in neuroblastoma cells. J Exp Clin Cancer Res 2009;28:19.
21. Li J, Yang L, Song L, et al. Astrocyte elevated gene-1 is a proliferation promoter in breast cancer via suppressing transcriptional factor FOXO1. Oncogene 2009;28:3188–96.
22. Lee SG, Su ZZ, Emdad L, Sarkar D, Franke TF, Fisher PB. Astrocyte elevated gene-1 activates cell survival pathways through PI3K-Akt signaling. Oncogene 2008;27:1114–21.
23. Khuda II-E, Koide N, Noman ASM, et al. Astrocyte elevated gene-1 (AEG-1) is induced by lipopolysaccharide as toll-like receptor 4 (TLR4) ligand and regulates TLR4 signaling. Immunology 2009;128:e700–6.
24. Lee SG, Su ZZ, Emdad L, Sarkar D, Fisher PB. Astrocyte elevated gene-1 (AEG-1) is a target gene of oncogenic Ha-ras requiring phosphatidylinositol 3-kinase and c-Myc. Proc Natl Acad Sci U S A 2006;103:17390–5.
25. Yoo BK, Gredler R, Vozhilla N, et al. Identification of genes conferring resistance to 5-fluorouracil. Proc Natl Acad Sci U S A 2009;106:12938–43.
26. Ash SC, Yang DQ, Britt DE. LYRIC/AEG-1 overexpression modulates BCCIPα protein levels in prostate tumor cells. Biochem Biophys Res Commun 2008;371:333–8.

Cancer Research
The Journal of Cancer Research (1916–1930) | The American Journal of Cancer (1931–1940)

AACR American Association for Cancer Research

# Astrocyte Elevated Gene-1: Far More Than Just a Gene Regulated in Astrocytes

Devanand Sarkar, Luni Emdad, Seok-Geun Lee, et al.

*Cancer Res* 2009;69:8529-8535. Published OnlineFirst November 10, 2009.