Pharmacology & Therapeutics 130 (2011) 1–8

Contents lists available at ScienceDirect

Pharmacology & Therapeutics

journal homepage: www.elsevier.com/locate/pharmthera

Associate Editor: P. Dent

Astrocyte elevated gene-1 (AEG-1): A multifunctional regulator of normal and abnormal physiology

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ARTICLE INFO

Keywords:
Astrocyte elevated gene-1 (AEG-1)
Oncogene
Metastasis
Chemoresistance
Angiogenesis
Neurodegeneration

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's 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.

Contents

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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0163-7258/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.pharmthera.2011.01.008

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 depends 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 & 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; Li et al., 2008; Lee et al., 2009; Hu et al., 2009; Yoo et al., 2009a; Song et al., 2009; Emdad et al., 2010; Song et al., 2010; Xia et al., 2010; Yu et al., 2009; Chen et al., 2010; Xu et al., in press) (Fig. 1). 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 bases 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., 2003, 2002). 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) (Fig. 2). 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., 2009a). 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., 2009a). AEG-1 is also detected in the ER/nuclear envelop (Sutherland et al., 2004; Kang et al., 2005) 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 Kinases 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., 2009a).

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 the skeletal muscle and the heart and in the endocrine glands such as the thyroid and the 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,