Abstract. Hepatocellular carcinoma (HCC) is the fifth most common malignancy worldwide, yet effective treatment for this disease is lacking. Thus, there is an urgent need to identify novel therapeutic targets for this dreadful disease. Numerous studies have established that overexpression of astrocyte-elevated gene-1 (AEG-1) is frequently observed in multiple types of cancers including HCC, and its expression levels are correlated with the stage and grade of the disease. Further studies revealed that AEG-1 plays a key role in several crucial aspects of HCC progression, including growth, transformation, cell survival, invasion, metastasis and chemoresistance. Moreover, AEG-1 overexpression activates the Wnt/β-catenin, mitogen-activated protein kinase (MAPK), nuclear factor (NF)-κB, and PI3K/Akt signaling pathways, and promotes its downstream gene expression to facilitate malignant potential. Recently, transgenic mice with hepatocyte-specific expression of AEG-1 (Alb/AEG-1) and AEG-1-knockout mouse both revealed novel aspects of the functions of AEG-1 in an in vivo context. This review evaluates the multi-functions of AEG-1 and describes the major signaling pathways and molecular alterations regulated by AEG-1 in HCC, indicating its key roles and potential as a biomarker or significant target for the therapy of HCC.

Contents

1. Background
2. Cloning and molecular characteristics of AEG-1
3. AEG-1 is overexpressed in HCC
4. Functions of AEG-1
5. Signaling pathways in HCC associated with AEG-1
6. AEG-1 as a potential biomarker and therapy for HCC
7. Conclusion

1. Background

Hepatocellular carcinoma (HCC) is the fifth (average of men and women) most common tumor and the third leading cause of cancer-related mortality worldwide (1). HCC is characterized by rapid growth, early vascular invasion, high-grade malignant potential and multidrug resistance (1-3). Although advances in treatment have contributed to improved survival, the overall 5-year survival remains less then 25% (4). Multiple etiologies, such as aflatoxin (5) as well as hepatitis B virus (HBV) (6) and hepatitis C virus (HCV) (7) infections, have been linked to HCC, while no consistent genetic abnormalities have been attributed to this disease. Numerous mutated proto-oncogenes and tumor-suppressor genes, as well as signaling pathway abnormalities have been detected in HCC such as p53, p73, Rb, adenomatous polyposis coli (APC), DLC-1, DLC-2, PTEN, SOCS1, GSTP1, HCCS1, Smad2/4, AXIN1, IGF-2, β-catenin, c-myc and cyclin D1 (8-10), which has hindered the development of effective targeted therapies. Therefore, identification of novel critical molecules that contribute to the progression of HCC would be extremely beneficial, not only for diagnostic/prognostic purposes but also for providing significant targets for therapeutic intervention. Within the last decade, overexpression of astrocyte-elevated gene-1 (AEG-1), a novel oncogene, also known as metadherin (MTDH) and lysine-rich CEACAM1 co-isolated (LYRIC), has been detected in the majority of cancers studied to date (11-13), and its elevated levels are associated with poor prognosis in cancer patients (14,15). In HCC, AEG-1 has been described as an essential gene involved in its progression (16).

2. Cloning and molecular characteristics of AEG-1

AEG-1 was originally reported as a novel late response gene induced in human fetal astrocytes after HIV-1 infection or treatment with viral glycoprotein gp120 or TNF-α (17). Subsequently, in vivo phage screening allowed the cloning of mouse AEG-1 as a protein mediating the metastasis of
breast cancer cells to the lung and was named metadherin (MTDH) (18). Mouse/rat AEG-1 was also cloned as a tight junction protein named LYRIC (19) and by gene trapping techniques and was named 3D3/LYRIC (20).

AEG-1 orthologues are found in most (over 90%) vertebrate species but not in non-vertebrates. The human AEG-1 gene is located on chromosome 8q22 having 12 exons/11 introns, and its genomic amplification has been detected in HCC and breast cancer (21). The human AEG-1 gene encodes a 582-amino acid protein with a calculated molecular mass of 64 kDa, and is present in the cell membrane, cytoplasm, nucleus, nucleolus and endoplasmic reticulum (20,22). It contains a transmembrane domain and three putative nuclear localization signals between amino acids 79-91, 432-451 and 561-580 (23). AEG-1 is ubiquitously lowly expressed in all normal tissues, with higher expression detected in the skeletal muscle and heart and in endocrine glands such as the thyroid and adrenal gland (22). AEG-1 is markedly upregulated in HCC (16,24), breast (25), gastric (26), gallbladder (27), colorectal (28), prostate (23) and renal (29) cancer, esophageal squamous cell carcinoma (ESSC) (30), non-small cell lung cancer (NSCLC) (31), pancreatic ductal adenocarcinoma (32), tongue carcinoma (33), melanoma (22), glioblastoma multiforme (GBM) (34), acute myeloid leukemia (35), neuroblastoma (36), oligodendroglioma (37) and osteosarcoma (38), cervical (39) and ovarian carcinoma (40).

3. AEG-1 is overexpressed in HCC

Numerous studies have documented that AEG-1 is overexpressed in HCC and is closely associated with the disease. In the earliest study by Yoo et al (16), among 109 HCC samples, only 7 scored negative for AEG-1 and the remaining 102 (93.58%) showed variable overexpression levels of AEG-1. Based on the Barcelona Clinic Liver Cancer (BCLC) staging system, the expression of AEG-1 is gradually increased with stages from I to IV, and a statistically significant correlation ($P<0.0001$) was obtained between the AEG-1 expression level and the stage of HCC (16). Our research team also found that AEG-1 was upregulated in HCC tissues among 60 pairs of HCC samples (41).

In a subsequent study, AEG-1 expression was assessed by immunohistochemistry in tissue microarrays of 323 HCC patients, which demonstrated that the majority of the tumor tissues expressed significantly higher levels of AEG-1 when compared with adjacent non-tumor tissues; with AEG-1$^{\text{High}}$ present in 54.2% (175 of 323) of all the patients (42). In addition, by Pearson $\chi^2$ test, AEG-1 expression was found to be closely associated with microvascular invasion ($P<0.001$), pathologic satellites ($P=0.007$), tumor differentiation ($P=0.002$) and TNM stage ($P=0.001$). Moreover, according to a cohort study, the 1-, 3- and 5-year overall survival (OS) rates in a high AEG-1-expressing group were significantly lower than those in a low AEG-1-expressing group (83.0 vs. 89.7%, 52.0 vs. 75.3% and 37.4 vs. 66.9%, respectively); the 1-, 3- and 5-year cumulative recurrence rates were markedly higher in the high AEG-1-expressing group than those in the low AEG-1-expressing group (32.4 vs. 16.8%, 61.2 vs. 38.2% and 70.7 vs. 47.8%, respectively). Furthermore, univariate and multivariate analyses revealed that along with tumor diameter, encapsulation, microvascular invasion and TNM stage, AEG-1 was an independent prognostic factor for both OS ($HR=1.870; P<0.001$) and recurrence ($HR=1.695; P<0.001$). Therefore, the overexpression of AEG-1 in HCC may predict shorter OS and a higher recurrence rate, and further become a marker for prognosis in HCC. In a more recent study in China in 89 human HCC patients, Zheng et al (43) also confirmed the above results.

In another separate study, AEG-1 expression levels were identified to be elevated in HBV-related HCC tissues ($n=73$) compared to normal liver tissues ($n=11$) or hepatitis samples ($n=45$), and were found to be correlated with the American Joint Committee on Cancer (AJCC, 7th edition) stage ($P=0.020$), T classification ($P=0.007$), N classification ($P=0.044$), vascular invasion ($P=0.006$) and histological differentiation ($P=0.020$) in patients with HBV-associated HCC (44). Moreover, patients with high AEG-1 levels had shorter survival times compared to those with low AEG-1 expression ($P=0.001$) (44). Additionally, expression of AEG-1 in HCV-related HCC was also significantly increased in comparison with the expression level in normal liver and cirrhotic tissue (16).

Taken together, AEG-1 overexpression is consistently observed in HCC, and its level appears to be correlated with the stage and grade as well as OS and the recurrence rate of HCC cases.

4. Functions of AEG-1

In parallel with the evaluation of the overexpression of AEG-1 in HCC, a substantial body of research has also highlighted the functions of AEG-1 in mediating the growth, metastasis and chemoresistance of the disease.

**AEG-1 accelerates the growth of HCC.** The most fundamental trait of cancer cells involves their ability to sustain proliferation (45). Previous studies as well as our study manipulating AEG-1 expression in HCC cells showed that overexpression of AEG-1 promotes proliferation and increases anchorage-independent growth in soft agar (16,46); knockdown of AEG-1 was found to suppress proliferation and inhibit colony formation as well as induce apoptosis through suppression of IL-6 secretion (47,48). Further studies also demonstrated that enhanced AEG-1 expression in HCC cells generated large and highly vascular subcutaneous tumors compared to the control; correspondingly, downregulation of the expression of AEG-1 decreased the tumor formation rate and the growth of subcutaneous tumors in nude mice and the tumor volumes were found to be smaller than the control (16,47). Additionally, transgenic mice with hepatocyte-specific expression of AEG-1 (Alb/AEG-1) were treated with N-nitrosodimethylamine, a hepatocarcinogen. A significant increase in the ratio of liver weight to body weight and the presence of more nodules of different sizes were noted in the Alb/AEG-1 mice when compared to these parameters in the WT mice (49). Based on the above studies, we regard AEG-1 as an accelerator of the growth of HCC.

**AEG-1 facilitates the metastasis of HCC.** Metastasis is not only the major cause of death from HCC, but is also the main obstacle to improving the prognosis of HCC (50,51). In a study...
using cell lines and a nude mouse model, downregulation of AEG-1 resulted in the reduced migratory capacity of HCC cell lines, as well as a reduction in pulmonary and abdominal metastases in mice (42). It was further demonstrated that the expression level of AEG-1 was correlated with four epithelial-to-mesenchymal transition (EMT) markers. Knockdown of AEG-1 expression level of AEG-1 was correlated with four epithelial-mesenchymal transition (EMT) markers. Knockdown of AEG-1 inhibited the metastatic potential of HCC cells through EMT (52). Apart from EMT, anoikis resistance is another important capacity to assess tumor metastatic potential, and is a prerequisite for the survival of circulating tumor cells in tumor metastasis (45,53). Our laboratory also demonstrated that AEG-1 enhanced the anoikis resistance in HCC cells through activation of the phosphatidylinositol 3-kinase (PI3K)/Akt pathway and Bcl family proteins to facilitate the metastasis of HCC (54).

Metastasis is a multistep biological process. In addition to EMT and anoikis resistance, there are other steps facilitated by AEG-1. Our laboratory found that AEG-1 conferred orientation chemotaxis to human pulmonary microvascular endothelial cells (HPMECs) mediated by CXCR4/CXCL12 in HCC cells (54). Moreover, overexpression of AEG-1 was found to lead to increased production of angiogenic factors, such as vascular endothelial growth factor (VEGF), placental growth factor (PIGF) and fibroblast growth factor-α (FGFα) in human HCC cells, which are essential for angiogenesis and metastasis (16). In addition, Alb/AEG-1 mice treated with N-nitrosodiethylamine, presented with multinodular HCC with steatotic features and associated modulation of expression of genes regulating invasion and metastasis (TSPAN8 and Lcn2) (49). In conclusion, AEG-1 promotes the metastasis of HCC by multiple steps, and plays a pivotal role in the poor prognosis of HCC. Thus, suppression of AEG-1 may be used as a candidate target therapy for HCC.

**AEG-1 promotes the chemoresistance of HCC.** Chemoresistance is an important hallmark of HCC. Recent studies have documented that AEG-1 contributes to broad-spectrum resistance to various chemotherapeutics including 5-fluorouracil (5-FU), doxorubicin, paclitaxel, cisplatin and 4-hydroxycyclophosphamide (4-HC) (16,21,55-57). Hepatocytes isolated from Alb/AEG-1 mice also displayed profound resistance to chemotherapeutics (49). Furthermore, microarray analysis of HCC revealed that AEG-1 upregulated several genes implicated in chemoresistance including drug-metabolizing enzymes, such as dihydroprymidine dehydrogenase (DPYD), cytochrome P450B6 (CYP2B6) and dihydroidol dehydrogenase (ARK1C2), ATP-binding cassette transporter ABCC11/MRP8 and the transcription factor LSF/TFCP2 (16). Moreover, AEG-1 was also found to facilitate the association of multidrug resistance gene (MDR) 1 mRNA to polysomes resulting in increased translation and inhibition of ubiquitination and subsequent proteasome-mediated degradation of MDR1 protein (56). Therefore, AEG-1 may promote chemoresistance through facilitating expression of drug-resistant genes at the transcription and translation levels and the attenuation of the drugs.

**5. Signaling pathways in HCC associated with AEG-1**

Over the past several decades, a large body of knowledge has been collected regarding Wnt/β-catenin, mitogen-activated protein kinase (MAPK), NF-κB and PI3K/Akt signaling pathways as the major signaling pathways activated in HCC (58-61). These signaling pathways have been demonstrated as being directly downstream of AEG-1. In addition, AEG-1 is also subtly regulated by its upstream, such as Ha-ras and miR-375.

**Upstream of AEG-1**

AEG-1 is reported as a downstream target of Ha-ras, and Ha-ras increases the binding of c-Myc to the E-box elements in the AEG-1 promoter through the PI3K/Akt/GSK3β/c-Myc pathway, which contributes to Ha-ras-mediated oncogenesis through AEG-1 (62). AEG-1 overexpression is also associated with elevated copy numbers of it, predominantly due to gains of large regions of chromosome 8q in HCC (16). We also demonstrated that miR-375 suppressed AEG-1 expression by binding directly to the 3'-UTR of AEG-1, and the negative regulation of AEG-1 by miR-375 may contribute partially to the antitumor effects of miR-375 involved in HCC. Thus, miR-375 is an important regulator of AEG-1 (41).

**Downstream of AEG-1**

Wnt/β-catenin and MAPK signaling pathways. Increasing evidence indicates that activation of the WNT/β-catenin-mediated signaling cascade plays a key role in hepatic oncogenesis (59,63). The transcription factor LEF-1, the ultimate executor of the Wnt pathway, heterodimerizes with β-catenin for its action. In the absence of Wnts, β-catenin is phosphorylated by GSK3β. Conversely, when Wnts are secreted, they can bind FZD and LRPS5/6, which leads to inactivation of GSK3β by phosphorylation, therefore increasing nuclear translocation of β-catenin to activate gene transcription such as c-Myc, cyclin D1, and members of the WISP family (64), facilitating the development of HCC. The Wnt pathway is activated by AEG-1 in the following ways (16).

i) AEG-1 directly induces expression of LEF-1 itself as well as LEF-1-induced genes. ii) By indirectly activating ERK42/44, AEG-1 leads to GSK3β phosphorylation and inactivation resulting in nuclear translocation of β-catenin. (iii) AEG-1 downregulates negative regulators of the Wnt pathway, such as APC and CTBP2.

Aberrant activation of the MAPK pathway also plays a critical role in the development and progression of HCC (65,66). Analysis of signal transduction pathways revealed activation of ERK42/44 and p38 MAPK in Hep-AEG-1 clones compared to control Hep-pc-4 clones. Inhibition of ERK42/44 and p38 MAPK pathways by their specific inhibitors PD98059 and SB203580, respectively, significantly inhibited AEG-1 induced Matrigel inhibition and anchorage-independent growth, but did not significantly affect increased proliferation, which indicates that the MEK/ERK and p38 MAPK pathways might mediate a more aggressive phenotype conferred by AEG-1 (16). Furthermore, activation of ERK42/44 above through interacting with GSK3β, crosstalks with the Wnt signaling pathway (16). That is to say, through two different manners, AEG-1 phosphorylates GSK3β to activate gene transcription. Additionally, AEG-1 also induces phosphoryla-
tion and inactivation of retinoid X receptor by activating ERK and p38MAPK signaling, which is indispensable to drive the oncogenic functions of HCC such as proliferation and apoptosis (67).

**NF-κB and phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathways.** NF-κB was found to be the first gene activated by AEG-1 (68,69). Its activation has been attributed to the acquisition of a transformed phenotype during hepatocarcinogenesis of HBV or HCV infection (70-72). An NF-κB luciferase reporter assay revealed an ~3-fold increase in basal activity and an ~5-fold increase in TNF-α-induced activity in Hep-AEG1-14 clones compared with the control, and similar findings were also observed in Hep-AEG1-8 clones (16).

Subsequently, a recent study using AEG-1-deficient mice found that AEG-1-deficient hepatocytes and macrophages exhibited a relative defect in NF-κB activation; and the IL-6 production and STAT-3 activation were also deficient along with other biological and epigenetic findings in the tumor microenvironment. This demonstrated that AEG-1 supports an NF-κB-mediated inflammatory state that drives HCC development (73).

Another signaling pathway modulated by AEG-1 is the PI3K/Akt pathway in HCC (74). As mentioned above, AEG-1 is regulated by Ha-ras through the PI3K/Akt/GSK3β/c-Myc pathway (62) in HCC. AEG-1 is transcriptionally regulated by c-Myc, and cooperates with c-Myc maternally imprinted non-coding RNAs, such as Rian, Meg-3 and Migr, which are implicated in the promotion of hepatocarcinogenesis (46).

AEG-1-dependent anoikis resistance is also activated via the PI3K/Akt pathway and Bcl-2, and the PI3K inhibitor AEG-1-dependent anoikis resistance was also confirmed by similar findings in the tumor microenvironment. This demonstrated that AEG-1 supports an NF-κB-mediated inflammatory state that drives HCC development (73).

**AEG-1-interacting protein, SND1.** Staphylococcal nuclease domain-containing 1 (SND1) is a multifunctional protein modulating a variety of cellular processes such as transcription (81,82), RNA splicing (83) and RNA metabolism (84). SND1 is overexpressed in HCC (85); knockdown of SND1 leads to reduced HCC cell proliferation, clone formation and tumor formation in nude mice (86); and it also regulates HCC angiogenesis by activation of NF-κB and miR-221 inducing angiogenic factors such as angiogenin and CXCL16 (87). The identification of SND1 as an AEG-1-interacting protein was found using two independent approaches, including yeast two hybrid screening using a human liver cDNA library and isolation of AEG-1 interacting proteins by co-immunoprecipitation followed by mass spectrometry. Moreover, immunofluorescence and co-immunoprecipitation analyses further demonstrated that AEG-1 interacts with SND1 via the region 101-205 a.a. in the cytoplasm (85). It was also documented that both AEG-1 and SND1 are required for optimum RNA-induced silencing complex (RISC) activity (85). Moreover, increased RISC activity, conferred by AEG-1 or SND1, was found to result in increased degradation of tumor-suppressor mRNAs, which are the target of oncomiRs, including PTEN (target of miR-221 and miR-21), CDKN1C (target of miR-221), CDKN1A (target of miR-106b), SPRY2 (target of miR-21) and TGFBR2 (target of miR-93) (85).

**Downstream genes of AEG-1 in HCC**

The transcription factor LSF/TFCP2 and IGFBP7. LSF, an ubiquitous transcription factor, has been demonstrated to function as an oncogene in HCC (75). It is highly expressed in HCC, and transcriptionally modulates specific genes, such as metalloproteinase-9 (MMP-9), c-Met and osteopontin (OPN), resulting in the regulation of proliferation, invasion, angiogenesis and chemoresistance of HCC (76,77). LSF has been identified as an AEG-1 downstream gene by Affymetrix microarray comparing global gene expression profiles between AEG-1-overexpressed clones of HepG3 cells and controls, which was also confirmed by TaqMan quantitative PCR (16). A subsequent study reported that LSF mRNA expression was ~15-fold higher in AEG-1-overexpressed clones compared to a control, which also indicates a potential role of LSF in mediating the oncogenic functions of AEG-1 (75).

IGFBP7, a secreted protein belonging to the IGFBP family, functions as a potential tumor suppressor in HCC (78). Multiple studies have documented that IGFBP7 expression is significantly decreased in HCC (79,80), and it profoundly decreases the viability and induces apoptosis in multiple human HCC cell lines and inhibits primary tumor growth and intrahepatic metastasis in orthotopic xenograft models (78). Notably, IGFBP7 has been identified as the most robustly downregulated gene by AEG-1 in HCC (16). Another study also demonstrated that stable IGFBP7-overexpressing clones were established in Hep-AEG1-14 background, and forced overexpression of IGFBP7 in AEG-1-overexpressing HCC cells inhibited in vitro growth and induced senescence, and profoundly suppressed in vivo growth in nude mice (79). Thus, mediated by LSF and IGFBP7, AEG-1 plays an important role in HCC progression.

**6. AEG-1 as a potential biomarker and therapy for HCC**

Since AEG-1 is markedly overexpressed in HCC tissues and its levels are tightly correlated with the stage and grade as well as the OS and recurrence rate of the disease, it might serve as a potential diagnostic/prognostic marker for HCC. In addition to HCC, in breast cancer, prostate cancer, ESSC, NSCLC, some subtypes of brain cancer such as GBM, and colorectal carcinoma, AEG-1 expression is also correlated with the stage or outcome of these diseases (23,25,30,31,34,88). Thus, AEG-1 may be a universal diagnostic/prognostic marker for cancer including HCC.

As known, HCC is a progressive and highly chemoresistant cancer, and there is no effective therapy for advanced
HCC. The only FDA-approved targeted drug, the multikinase inhibitor sorafenib, provides a survival benefit of only 2.8 months in non-resectable HCC patients (89). AEG-1 is a key molecule involved in several important signaling pathways which mediate the progression of HCC and is markedly overexpressed in HCC. Thus, specific inhibition of AEG-1 may be a strategy to counteract the progression of HCC. Moreover, as mentioned above, AEG-1 overexpression contributes to HCC drug resistance at multiple levels. Therefore, specific inhibition of AEG-1 not only blocks HCC progression, but also enhances the effect of anti-HCC drugs such as 5-FU. A combination of AEG-1 inhibitors with chemotherapeutics may be an effective treatment for HCC. A lentivirus delivering AEG-1 siRNA in combination with 5-FU was found to markedly inhibit the growth of QGY-7703 HCC cell xenografts in athymic nude mice when compared to either agent alone, and the combination treatment reduced the tumor volume and tumor weight ~70% compared to the control (55).

7. Conclusion

To date, it has been established that AEG-1 is frequently upregulated and functions as an oncogene by regulating several major signaling pathways in HCC as summarized in Fig. 1. Given the importance of AEG-1 in HCC carcinogenesis, it is not surprising that the potential clinical application of AEG-1 in HCC diagnosis and therapy warrants further investigation. In addition to tissue AEG-1, whether AEG-1 in blood, urine or other secretions is also associated with the stage and grade of HCC needs to be determined. Moreover, methods to detect these levels efficiently are vitally needed. Therefore, further studies using large cohorts of patients are warranted to resolve these issues. Moreover, there are still challenges in regards to the means of transport of AEG-1 inhibitors in the clinical therapy of HCC. A safe and effective carrier to deliver inhibitors of AEG-1 into HCC cells is needed. Recently, gold nanoparticles have demonstrated increasingly wide applications in drug delivery due to their unique physicochemical and optical properties as well as their low toxicity when compared to organic nanocarriers (90,91). These may be helpful as a new means of transport of AEG-1 inhibitors in clinical application.

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