The emerging role of astrocyte-elevated gene-1 in hepatocellular carcinoma (Review)

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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-actived 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.

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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

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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) andosteosarcoma (38), cervical (39) and ovarian carcinoma (40).

3. AEG-1 is overexpessed 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^{High} present in 54.2% (175 of 323) of all the patients (42). In addition, by Pearson χ^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-nitrosodiethylamine, 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 in HCC cell lines resulted in downregulation of N-cadherin and Snail, upregulation of E-cadherin and translocation of β-catenin (42). Another study using Huaier polysaccharide also confirmed that downregulation of AEG-1 inhibited the metastatic potential of HCC cells through EMT (52). Apart from EMT, anoikis resistance is an 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 dihydropryimidine dehydrogenase (DPYD), cytochrome P450B6 (CYP2B6) and dyhydrodiol 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-actived 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 LRP5/6, 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), faciliting 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 hepato-carcinogenesis 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 LY294002 was found to reverse the AEG-1-dependent effects on Akt phosphorylation, expression and anoikis resistance (54). Moreover, AEG-1 also activates the PI3K/Akt/mTOR pathway resulting in an increase in MDR1 levels by increased association of MDR1 mRNA to polysomes in drug-resistant human HCC cells (56).

Finally, AEG-1 regulates these major signaling pathways and plays a crucial role in the development of HCC. However, there is still a need to explore the network of AEG-1 in HCC, and AEG-1 also regulates the expression of many down-stream genes such as late SV40 factor (LSF), insulin-like growth factor binding protein-7 (IGFBP7) and participates in RISC by interacting with SND1, which acts to accelerate HCC.

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 indcates 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-AEG-1-14 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.

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 isola-tion 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).

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 carci-noma, 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 chemoresis-tant cancer, and there is no effective therapy for advanced
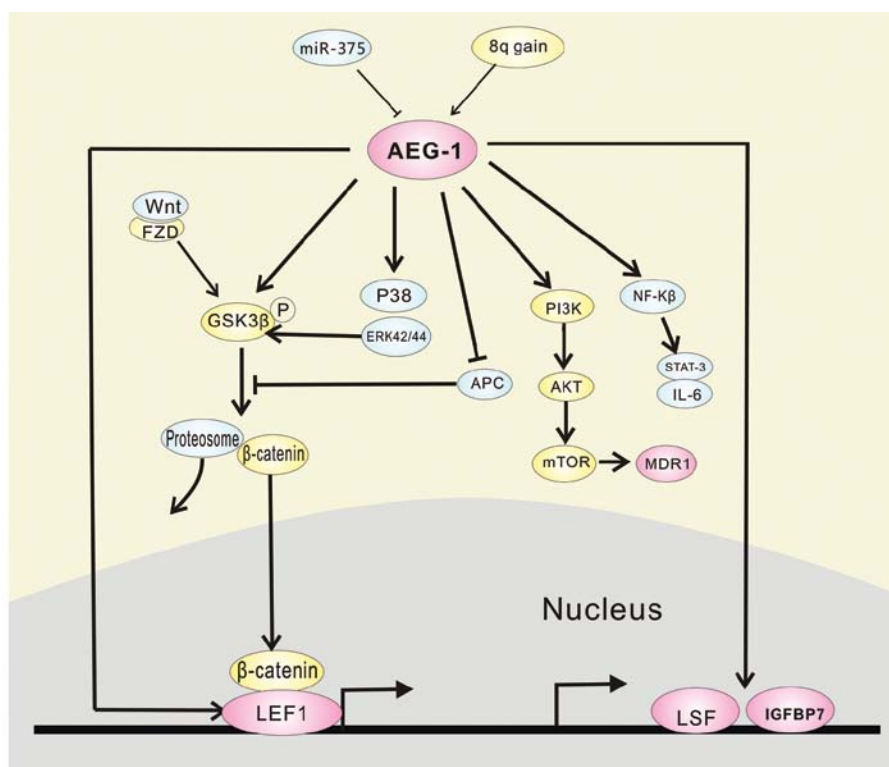
Figure 1. Molecular mechanism of AEG-1 action in HCC. Thick arrows indicate positive regulation by AEG-1 while thin arrow indicates regulation of AEG-1. '⊣' indicates negative regulation. AEG-1, astrocyte-elevated gene-1; HCC, hepatocellular carcinoma.

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.

References

1. El Serag HB: Hepatocellular carcinoma. N Engl J Med 365: 1118-1127, 2011.
2. Pang RW, Joh JW, Johnson PJ, Monden M, Pawlik TM and Poon RT: Biology of hepatocellular carcinoma. Ann Surg Oncol 15: 962-971, 2008.
3. El Serag HB and Rudolph KL: Hepatocellular carcinoma: Epidemiology and molecular carcinogenesis. Gastroenterology 132: 2557-2576, 2007.
4. Altekruse SF, McGlynn KA and Reichman ME: Hepatocellular carcinoma incidence, mortality, and survival trends in the United States from 1975 to 2005. J Clin Oncol 27: 1485-1491, 2009.

5. Hagymási K and Tulassay Z: Epidemiology, risk factors and molecular pathogenesis of primary liver cancer. Orv Hetil 149: 541-548, 2008 (In Hungarian).

6. Feitelson MA and Duan LX: Hepatitis B virus X antigen in the pathogenesis of chronic infections and the development of hepatocellular carcinoma. Am J Pathol 150: 1141-1157, 1997.

7. Majumder M, Ghosh AK, Steele R, Ray R and Ray RB: Hepatitis C virus NS5A physically associates with p53 and regulates p21/waf1 gene expression in a p53-dependent manner. J Virol 75: 1401-1407, 2001.

8. Boyault S, Rickman DS, de Reyniès A, Balabaud C, Rebouissou S, Jeannot E, Hérault A, Saric J, Belghiti J, Franco D, *et al*: Transcriptome classification of HCC is related to gene alterations and to new therapeutic targets. Hepatology 45: 42-52, 2007.

9. Katoh H, Ojima H, Kokubu A, Saito S, Kondo T, Kosuge T, Hosoda F, Imoto I, Inazawa J, Hirohashi S, *et al*: Genetically distinct and clinically relevant classification of hepatocellular carcinoma: Putative therapeutic targets. Gastroenterology 133: 1475-1486, 2007.

10. Mann CD, Neal CP, Garcea G, Manson MM, Dennison AR and Berry DP: Prognostic molecular markers in hepatocellular carcinoma: A systematic review. Eur J Cancer 43: 979-992, 2007.

11. Sarkar D and Fisher PB: AEG-1/MTDH/LYRIC: Clinical significance. Adv Cancer Res 120: 39-74, 2013.

12. Yoo BK, Emdad L, Lee SG, Su ZZ, Santhekadur P, Chen D, Gredler R, Fisher PB and Sarkar D: Astrocyte elevated gene-1 (AEG-1): A multifunctional regulator of normal and abnormal physiology. Pharmacol Ther 130: 1-8, 2011.

13. Lee SG, Kang DC, DeSalle R, Sarkar D and Fisher PB: AEG-1/MTDH/LYRIC, the beginning: Initial cloning, structure, expression profile, and regulation of expression. Adv Cancer Res 120: 1-38, 2013.

14. Wan L, Lu X, Yuan S, Wei Y, Guo F, Shen M, Yuan M, Chakrabarti R, Hua Y, Smith HA, *et al*: MTDH-SND1 interaction is crucial for expansion and activity of tumor-initiating cells in diverse oncogene- and carcinogen-induced mammary tumors. Cancer Cell 26: 92-105, 2014.

15. Ying Z, Li J and Li M: Astrocyte elevated gene 1: Biological functions and molecular mechanism in cancer and beyond. Cell Biosci 1: 36, 2011.

16. Yoo BK, Emdad L, Su ZZ, Villanueva A, Chiang DY, Mukhopadhyay ND, Mills AS, Waxman S, Fisher RA, Llovet JM, *et al*: Astrocyte elevated gene-1 regulates hepatocellular carcinoma development and progression. J Clin Invest 119: 465-477, 2009.

17. Su ZZ, Kang DC, Chen Y, Pekarskaya O, Chao W, Volsky DJ and Fisher PB: 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 21: 3592-3602, 2002.

18. Brown DM and Ruoslahti E: Metadherin, a cell surface protein in breast tumors that mediates lung metastasis. Cancer Cell 5: 365-374, 2004.

19. Britt DE, Yang DF, Yang DQ, Flanagan D, Callanan H, Lim YP, Lin SH and Hixson DC: Identification of a novel protein, LYRIC, localized to tight junctions of polarized epithelial cells. Exp Cell Res 300: 134-148, 2004.

20. Sutherland HG, Lam YW, Briers S, Lamond AI and 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 294: 94-105, 2004.

21. Hu G, Chong RA, Yang Q, Wei Y, Blanco MA, Li F, Reiss M, Au JL, Haffty BG and Kang Y: MTDH activation by 8q22 genomic gain promotes chemoresistance and metastasis of poor-prognosis breast cancer. Cancer Cell 15: 9-20, 2009.

22. Kang DC, Su ZZ, Sarkar D, Emdad L, Volsky DJ and Fisher PB: Cloning and characterization of HIV-1-inducible astrocyte elevated gene-1, AEG-1. Gene 353: 8-15, 2005.

23. Thirkettle HJ, Girling J, Warren AY, Mills IG, Sahadevan K, Leung H, Hamdy F, Whitaker HC and Neal DE: LYRIC/AEG-1 is targeted to different subcellular compartments by ubiquitinylation and intrinsic nuclear localization signals. Clin Cancer Res 15: 3003-3013, 2009.

24. Sarkar D: AEG-1/MTDH/LYRIC in liver cancer. Adv Cancer Res 120: 193-221, 2013.

25. Li J, Zhang N, Song LB, Liao WT, Jiang LL, Gong LY, Wu J, Yuan J, Zhang HZ, Zeng MS, *et al*: Astrocyte elevated gene-1 is a novel prognostic marker for breast cancer progression and overall patient survival. Clin Cancer Res 14: 3319-3326, 2008.

26. Jianbo X, Hui W, Yulong H, Changhua Z, Longjuan Z, Shirong C and Wenhua Z: Astrocyte-elevated gene-1 overexpression is associated with poor prognosis in gastric cancer. Med Oncol 28: 455-462, 2011.

27. Sun W, Fan YZ, Xi H, Lu XS, Ye C and Zhang JT: Astrocyte elevated gene-1 overexpression in human primary gallbladder carcinomas: An unfavorable and independent prognostic factor. Oncol Rep 26: 1133-1142, 2011.

28. Song HT, Qin Y, Yao GD, Tian ZN, Fu SB and Geng JS: Astrocyte elevated gene-1 mediates glycolysis and tumorigenesis in colorectal carcinoma cells via AMPK signaling. Mediators Inflamm 2014: 287381, 2014.

29. Chen W, Ke Z, Shi H, Yang S and Wang L: Overexpression of AEG-1 in renal cell carcinoma and its correlation with tumor nuclear grade and progression. Neoplasma 57: 522-529, 2010.

30. Yu C, Chen K, Zheng H, Guo X, Jia W, Li M, Zeng M, Li J and Song L: Overexpression of astrocyte elevated gene-1 (AEG-1) is associated with esophageal squamous cell carcinoma (ESCC) progression and pathogenesis. Carcinogenesis 30: 894-901, 2009.

31. Song L, Li W, Zhang H, Liao W, Dai T, Yu C, Ding X, Zhang L and Li J: Over-expression of AEG-1 significantly associates with tumour aggressiveness and poor prognosis in human non-small cell lung cancer. J Pathol 219: 317-326, 2009.

32. Huang Y, Ren GP, Xu C, Dong SF, Wang Y, Gan Y, Zhu L and Feng TY: Expression of astrocyte elevated gene-1 (AEG-1) as a biomarker for aggressive pancreatic ductal adenocarcinoma. BMC Cancer 14: 479, 2014.

33. Ke ZF, He S, Li S, Luo D, Feng C and Zhou W: Expression characteristics of astrocyte elevated gene-1 (AEG-1) in tongue carcinoma and its correlation with poor prognosis. Cancer Epidemiol 37: 179-185, 2013.

34. Emdad L, Sarkar D, Lee SG, Su ZZ, Yoo BK, Dash R, Yacoub A, Fuller CE, Shah K, Dent P, *et al*: Astrocyte elevated gene-1: A novel target for human glioma therapy. Mol Cancer Ther 9: 79-88, 2010.

35. Long M, Hao M, Dong K, Shen J, Wang X, Lin F, Liu L, Wei J, Liang Y, Yang J, *et al*: AEG-1 overexpression is essential for maintenance of malignant state in human AML cells via up-regulation of Akt1 mediated by AURKA activation. Cell Signal 25: 1438-1446, 2013.

36. Lee SG, Jeon HY, Su ZZ, Richards JE, Vozhilla N, Sarkar D, Van Maerken T and Fisher PB: Astrocyte elevated gene-1 contributes to the pathogenesis of neuroblastoma. Oncogene 28: 2476-2484, 2009.

37. Xia Z, Zhang N, Jin H, Yu Z, Xu G and Huang Z: Clinical significance of astrocyte elevated gene-1 expression in human oligodendrogliomas. Clin Neurol Neurosurg 112: 413-419, 2010.

38. Wang F, Ke ZF, Sun SJ, Chen WF, Yang SC, Li SH, Mao XP and Wang LT: Oncogenic roles of astrocyte elevated gene-1 (AEG-1) in osteosarcoma progression and prognosis. Cancer Biol Ther 12: 539-548, 2011.

39. Long M, Dong K, Gao P, Wang X, Liu L, Yang S, Lin F, Wei J and Zhang H: Overexpression of astrocyte-elevated gene-1 is associated with cervical carcinoma progression and angiogenesis. Oncol Rep 30: 1414-1422, 2013.

40. Li C, Chen K, Cai J, Shi QT, Li Y, Li L, Song H, Qiu H, Qin Y and Geng JS: Astrocyte elevated gene-1: A novel independent prognostic biomarker for metastatic ovarian tumors. Tumour Biol 35: 3079-3085, 2014.

41. He XX, Chang Y, Meng FY, Wang MY, Xie QH, Tang F, Li PY, Song YH and Lin JS: MicroRNA-375 targets AEG-1 in hepatocellular carcinoma and suppresses liver cancer cell growth in vitro and in vivo. Oncogene 31: 3357-3369, 2012.

42. Zhu K, Dai Z, Pan Q, Wang Z, Yang GH, Yu L, Ding ZB, Shi GM, Ke AW, Yang XR, *et al*: Metadherin promotes hepatocellular carcinoma metastasis through induction of epithelial-mesenchymal transition. Clin Cancer Res 17: 7294-7302, 2011.

43. Zheng J, Li C, Wu X, Yang Y, Hao M, Sheng S, Sun Y, Zhang H, Long J and Hu C: Astrocyte elevated gene-1 is a novel biomarker of epithelial-mesenchymal transition and progression of hepatocellular carcinoma in two China regions. Tumour Biol 35: 2265-2269, 2014.

44. Gong Z, Liu W, You N, Wang T, Wang X, Lu P, Zhao G, Yang P, Wang D and Dou K: Prognostic significance of metadherin overexpression in hepatitis B virus-related hepatocellular carcinoma. Oncol Rep 27: 2073-2079, 2012.

45. Hanahan D and Weinberg RA: Hallmarks of cancer: The next generation. Cell 144: 646-674, 2011.

46. Srivastava J, Siddiq A, Gredler R, Shen XN, Rajasekaran D, Robertson CL, Subler MA, Windle JJ, Dumur CI, Mukhopadhyay ND, et al: Astrocyte elevated gene-1 (AEG-1) and c-Myc cooperate to promote hepatocarcinogenesis. Hepatology 61: 915-929, 2014.

47. Deng H, Zhou Z, Tu W, Xia Y, Huang H and Tian D: Knockdown of astrocyte elevated gene-1 inhibits growth through suppression of IL-6 secretion in HepG2 human hepatoma cells. Oncol Lett 7: 101-106, 2014.

48. Ma J, Xie SL, Geng YJ, Jin S, Wang GY and Lv GY: In vitro regulation of hepatocellular carcinoma cell viability, apoptosis, invasion, and AEG-1 expression by LY294002. Clin Res Hepatol Gastroenterol 38: 73-80, 2014.

49. Srivastava J, Siddiq A, Emdad L, Santhekadur PK, Chen D, Gredler R, Shen XN, Robertson CL, Dumur CI, Hylemon PB, et al: Astrocyte elevated gene-1 promotes hepatocarcinogenesis: Novel insights from a mouse model. Hepatology 56: 1782-1791, 2012.

50. Tang ZY, Ye SL, Liu YK, Qin LX, Sun HC, Ye QH, Wang L, Zhou J, Qiu SJ, Li Y, et al: A decade's studies on metastasis of hepatocellular carcinoma. J Cancer Res Clin Oncol 130: 187-196, 2004.

51. Peng YF, Shi YH, Ding ZB, Ke AW, Gu CY, Hui B, Zhou J, Qiu SJ, Dai Z and Fan J: Autophagy inhibition suppresses pulmonary metastasis of HCC in mice via impairing anoikis resistance and colonization of HCC cells. Autophagy 9: 2056-2068, 2013.

52. Zheng J, Li C, Wu X, Liu M, Sun X, Yang Y, Hao M, Sheng S, Sun Y, Zhang H, et al: Huaier polysaccharides suppresses hepatocarcinoma MHCC97-H cell metastasis via inactivation of EMT and AEG-1 pathway. Int J Biol Macromol 64: 106-110, 2014.

53. Frisch SM and Screaton RA: Anoikis mechanisms. Curr Opin Cell Biol 13: 555-562, 2001.

54. Zhou Z, Deng H, Yan W, Luo M, Tu W, Xia Y, He J, Han P, Fu Y and Tian D: AEG-1 promotes anoikis resistance and orientation chemotaxis in hepatocellular carcinoma cells. PLoS One 9: e100372, 2014.

55. Yoo BK, Gredler R, Vozhilla N, Su ZZ, Chen D, Forcier T, Shah K, Saxena U, Hansen U, Fisher PB, et al: Identification of genes conferring resistance to 5-fluorouracil. Proc Natl Acad Sci USA 106: 12938-12943, 2009.

56. Yoo BK, Chen D, Su ZZ, Gredler R, Yoo J, Shah K, Fisher PB and Sarkar D: Molecular mechanism of chemoresistance by astrocyte elevated gene-1. Cancer Res 70: 3249-3258, 2010.

57. Liu H, Song X, Liu C, Xie L, Wei L and 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 28: 19, 2009.

58. Min L, He B and Hui L: Mitogen-activated protein kinases in hepatocellular carcinoma development. Semin Cancer Biol 21: 10-20, 2011.

59. Thompson MD and Monga SP: WNT/beta-catenin signaling in liver health and disease. Hepatology 45: 1298-1305, 2007.

60. Villanueva A, Chiang DY, Newell P, Peix J, Thung S, Alsinet C, Tovar V, Roayaie S, Minguez B, Sole M, et al: Pivotal role of mTOR signaling in hepatocellular carcinoma. Gastroenterology 135: 1972-1983, 2008.

61. Pikarsky E, Porat RM, Stein I, Abramovitch R, Amit S, Kasem S, Gutkovich-Pyest E, Urieli-Shoval S, Galun E and Ben-Neriah Y: NF-kappaB functions as a tumour promoter in inflammation-associated cancer. Nature 431: 461-466, 2004.

62. Lee SG, Su ZZ, Emdad L, Sarkar D and 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 USA 103: 17390-17395, 2006.

63. Wands JR and Kim M: WNT/β-catenin signaling and hepatocellular carcinoma. Hepatology 60: 452-454, 2014.

64. Tanaka S, Sugimachi K, Kameyama T, Maehara S, Shirabe K, Shimada M, Wands JR and Maehara Y: Human WISP1v, a member of the CCN family, is associated with invasive cholangiocarcinoma. Hepatology 37: 1122-1129, 2003.

65. Wang S, Huang X, Li Y, Lao H, Zhang Y, Dong H, Xu W, Li JL and Li M: RN181 suppresses hepatocellular carcinoma growth by inhibition of the ERK/MAPK pathway. Hepatology 53: 1932-1942, 2011.

66. Guichard C, Amaddeo G, Imbeaud S, Ladeiro Y, Pelletier L, Maad IB, Calderaro J, Bioulac-Sage P, Letexier M, Degos F, et al: Integrated analysis of somatic mutations and focal copy-number changes identifies key genes and pathways in hepatocellular carcinoma. Nat Genet 44: 694-698, 2012.

67. Srivastava J, Robertson CL, Rajasekaran D, Gredler R, Siddiq A, Emdad L, Mukhopadhyay ND, Ghosh S, Hylemon PB, Gil G, et al: AEG-1 regulates retinoid X receptor and inhibits retinoid signaling. Cancer Res 74: 4364-4377, 2014.

68. Emdad L, Sarkar D, Su ZZ, Randolph A, Boukerche H, Valerie K and Fisher PB: Activation of the nuclear factor κB pathway by astrocyte elevated gene-1: Implications for tumor progression and metastasis. Cancer Res 66: 1509-1516, 2006.

69. Sarkar D, Park ES, Emdad L, Lee SG, Su ZZ and Fisher PB: Molecular basis of nuclear factor-κB activation by astrocyte elevated gene-1. Cancer Res 68: 1478-1484, 2008.

70. Hösel M, Quasdorff M, Wiegmann K, Webb D, Zedler U, Broxtermann M, Tedjokusumo R, Esser K, Arzberger S, Kirschning CJ, et al: Not interferon, but interleukin-6 controls early gene expression in hepatitis B virus infection. Hepatology 50: 1773-1782, 2009.

71. Tai DI, Tsai SL, Chang YH, Huang SN, Chen TC, Chang KS and Liaw YF: Constitutive activation of nuclear factor κB in hepatocellular carcinoma. Cancer 89: 2274-2281, 2000.

72. Liu P, Kimmoun E, Legrand A, Sauvanet A, Degott C, Lardeux B and Bernuau D: Activation of NF-κB, AP-1 and STAT transcription factors is a frequent and early event in human hepatocellular carcinomas. J Hepatol 37: 63-71, 2002.

73. Robertson CL, Srivastava J, Siddiq A, Gredler R, Emdad L, Rajasekaran D, Akiel M, Shen XN, Guo C, Giashuddin S, et al: Genetic deletion of AEG-1 prevents hepatocarcinogenesis. Cancer Res 74: 6184-6193, 2014.

74. Grabinski N, Ewald F, Hofmann BT, Staufer K, Schumacher U, Nashan B and Jücker M: Combined targeting of AKT and mTOR synergistically inhibits proliferation of hepatocellular carcinoma cells. Mol Cancer 11: 85, 2012.

75. Yoo BK, Emdad L, Gredler R, Fuller C, Dumur CI, Jones KH, Jackson-Cook C, Su ZZ, Chen D, Saxena UH, et al: Transcription factor Late SV40 Factor (LSF) functions as an oncogene in hepatocellular carcinoma. Proc Natl Acad Sci USA 107: 8357-8362, 2010.

76. Santhekadur PK, Gredler R, Chen D, Siddiq A, Shen XN, Das SK, Emdad L, Fisher PB and Sarkar D: Late SV40 factor (LSF) enhances angiogenesis by transcriptionally up-regulating matrix metalloproteinase-9 (MMP-9). J Biol Chem 287: 3425-3432, 2012.

77. Yoo BK, Gredler R, Chen D, Santhekadur PK, Fisher PB and Sarkar D: c-Met activation through a novel pathway involving osteopontin mediates oncogenesis by the transcription factor LSF. J Hepatol 55: 1317-1324, 2011.

78. Chen D, Siddiq A, Emdad L, Rajasekaran D, Gredler R, Shen XN, Santhekadur PK, Srivastava J, Robertson CL, Dmitriev I, et al: Insulin-like growth factor-binding protein-7 (IGFBP7): A promising gene therapeutic for hepatocellular carcinoma (HCC). Mol Ther 21: 758-766, 2013.

79. Chen D, Yoo BK, Santhekadur PK, Gredler R, Bhutia SK, Das SK, Fuller C, Su ZZ, Fisher PB and Sarkar D: Insulin-like growth factor-binding protein-7 functions as a potential tumor suppressor in hepatocellular carcinoma. Clin Cancer Res 17: 6693-6701, 2011.

80. Tomimaru Y, Eguchi H, Wada H, Kobayashi S, Marubashi S, Tanemura M, Umeshita K, Kim T, Wakasa K, Doki Y, et al: IGFBP7 downregulation is associated with tumor progression and clinical outcome in hepatocellular carcinoma. Int J Cancer 130: 319-327, 2012.

81. Leverson JD, Koskinen PJ, Orrico FC, Rainio EM, Jalkanen KJ, Dash AB, Eisenman RN and Ness SA: Pim-1 kinase and p100 cooperate to enhance c-Myb activity. Mol Cell 2: 417-425, 1998.

82. Wang X, Liu X, Fang J, Lu Y, He J, Yao X, Yao Z and Yang J: Coactivator P100 protein enhances STAT6-dependent transcriptional activation but has no effect on STAT1-mediated gene transcription. Anat Rec 293: 1010-1016, 2010.

83. Gao X, Zhao X, Zhu Y, He J, Shao J, Su C, Zhang Y, Zhang W, Saarikettu J, Silvennoinen O, et al: Tudor staphylococcal nuclease (Tudor-SN) participates in small ribonucleoprotein (snRNP) assembly via interacting with symmetrically dimethylated Sm proteins. J Biol Chem 287: 18130-18141, 2012.

84. Gao X, Ge L, Shao J, Su C, Zhao H, Saarikettu J, Yao X, Yao Z, Silvennoinen O and Yang J: Tudor-SN interacts with and co-localizes with G3BP in stress granules under stress conditions. FEBS Lett 584: 3525-3532, 2010.

85. Yoo BK, Santhekadur PK, Gredler R, Chen D, Emdad L, Bhutia S, Pannell L, Fisher PB and Sarkar D: Increased RNA-induced silencing complex (RISC) activity contributes to hepatocellular carcinoma. Hepatology 53: 1538-1548, 2011.

86. Yin J, Ding J, Huang L, Tian X, Shi X, Zhi L, Song J, Zhang Y, Gao X, Yao Z, *et al*: SND1 affects proliferation of hepatocellular carcinoma cell line SMMC-7721 by regulating IGFBP3 expression. Anat Rec 296: 1568-1575, 2013.
87. Santhekadur PK, Das SK, Gredler R, Chen D, Srivastava J, Robertson C, Baldwin AS Jr, Fisher PB and Sarkar D: Multifunction protein staphylococcal nuclease domain containing 1 (SND1) promotes tumor angiogenesis in human hepatocellular carcinoma through novel pathway that involves nuclear factor κB and miR-221. J Biol Chem 287: 13952-13958, 2012.
88. Song H, Li C, Li R and Geng J: Prognostic significance of AEG-1 expression in colorectal carcinoma. Int J Colorectal Dis 25: 1201-1209, 2010.
89. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, Santoro A, Raoul JL, Forner A, *et al*: SHARP Investigators Study Group: Sorafenib in advanced hepatocellular carcinoma. N Engl J Med 359: 378-390, 2008.
90. Liang JJ, Zhou YY, Wu J and Ding Y: Gold nanoparticle-based drug delivery platform for antineoplastic chemotherapy. Curr Drug Metab 15: 620-631, 2014.
91. Dykman LA and Khlebtsov NG: Uptake of engineered gold nanoparticles into mammalian cells. Chem Rev 114: 1258-1288, 2014.