Astrocyte Elevated Gene-1 Promotes Hepatocarcinogenesis: Novel Insights From a Mouse Model

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Astrocyte elevated gene-1 (AEG-1) is a key contributor to hepatocellular carcinoma (HCC) development and progression. To enhance our understanding of the role of AEG-1 in hepatocarcinogenesis, a transgenic mouse with hepatocyte-specific expression of AEG-1 (Alb/AEG1) was developed. Treating Alb/AEG-1, but not wild-type (WT) mice, with N-nitrosodiethylamine resulted in multinodular HCC with steatotic features and associated modulation of expression of genes regulating invasion, metastasis, angiogenesis, and fatty acid synthesis. Hepatocytes isolated from Alb/AEG-1 mice displayed profound resistance to chemothapeutics and growth factor deprivation with activation of prosurvival signaling pathways. Alb/AEG-1 hepatocytes also exhibited marked resistance toward senescence, which correlated with abrogation of activation of a DNA damage response. Conditioned media from Alb/AEG-1 hepatocytes induced marked angiogenesis with elevation in several coagulation factors. Among these factors, AEG-1 facilitated the association of factor XII (FXII) messenger RNA with polysomes, resulting in increased translation. Short interfering RNA–mediated knockdown of FXII resulted in profound inhibition of AEG-1-induced angiogenesis. Conclusion: We uncovered novel aspects of AEG-1 functions, including induction of steatosis, inhibition of senescence, and activation of the coagulation pathway to augment aggressive hepatocarcinogenesis. The Alb/AEG-1 mouse provides an appropriate model to scrutinize the molecular mechanism of hepatocarcinogenesis and to evaluate the efficacy of novel therapeutic strategies targeting HCC. (Hepatology 2012;56:1782-1791)

Abbreviations: Alb, antibodies; AEG-1, astrocyte elevated gene-1; Akt, protein kinase B; ALB, albumin; Alb/AEG1, transgenic mouse with hepatocyte-specific expression of AEG-1; ATM, ataxia telangiectasia mutated; CAM, chicken chorioallantoic membrane; CBP, CREB-binding protein; CM, conditioned media; CoA, coenzyme A; Cs, cycle threshold; DEN, N-nitrosodiethylamine; DOX, doxorubicin; ECs, endothelial cells; EGFR, epidermal growth factor receptor; ERK, extracellular signal-related kinase; 5-FU, 5-fluorouracil; FXII, coagulation factor XII; HCC, hepatocellular carcinoma; HUVECs, human vascular endothelial cells; IHC, immunohistochemistry; IP, intraperitoneal; KO, knockout; LC, liquid chromatography; MAPK, mitogen-activated protein kinase; MDR1, multidrug resistance protein 1; mRNA, messenger RNA; miRNA, microRNA; MS, mass spectrometry; MTT, tetrazolium; NAFLD, nonalcoholic fatty liver disease; NF-kB, nuclear factor κappa light-chain enhancer of activated B cells; PCR, polymerase chain reaction; ROS, reactive oxygen species; SA-β-gal, senescence-associated β-galactosidase; SAHF, senescence-associated heterochromatin foci; SCD, stearoyl coenzyme A desaturase; SEM, standard error of the mean; siRNA, short interfering RNA; TFF3, trefoil factor 3; TG, transgenic; WT, wild type.

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Received January 26, 2012; accepted May 18, 2012.

The present study was supported, in part, by grants from the James S. McDonnell Foundation and National Cancer Institute (grant no.: R01 CA138540; to D.S.), the Samuel Waxman Cancer Research Foundation (SWCRF; grant to D.S. and P.B.F.), and the National Institutes of Health (grant no.: R01 CA134721; to P.B.F.). D.S. is the Harrison Endowed Scholar in Cancer Research and a Blick scholar. P.B.F holds the Thelma Newman Corman Chair in Cancer Research and is a SWCRF investigator.
displays a negative correlation with patient survival. The AEG-1 gene is located in human chromosome 8q22, which is amplified in breast and liver cancers.\textsuperscript{2,3} AEG-1 is a downstream gene in the Ha-Ras-signaling pathway that activates phosphoinositol 3-kinase/protein kinase B (Akt) and leads to transcriptional up-regulation of AEG-1 by c-Myc.\textsuperscript{4} AEG-1 is a target of microRNA (miRNA)-375, a tumor suppressor in diverse cancers.\textsuperscript{5} Thus, AEG-1 expression might be increased by a variety of mechanisms during carcinogenesis.

Gain- and loss-of-function studies in diverse cell lines confirm the importance of AEG-1 in the development and progression of cancer. In multiple cancer cell lines that express low levels of AEG-1 and are poorly aggressive, AEG-1 overexpression results in a significant increase in \textit{in vitro} proliferation, anchorage-independent growth, migration and invasion and \textit{in vivo} tumorigenesis, metastasis, and angiogenesis in nude mouse xenograft models.\textsuperscript{1} As a corollary, RNA interference–mediated inhibition of AEG-1 in aggressive cell lines expressing high levels of AEG-1 significantly inhibits aforementioned \textit{in vitro} and \textit{in vivo} oncogenic phenotypes. AEG-1 overexpression results in the activation of multiple prosurvival signal-transduction pathways and profoundly contributes to chemoresistance and tumor angiogenesis, major hallmarks of aggressive cancers.\textsuperscript{1} Thus, AEG-1 plays a fundamental role in aggressive progression of the carcinogenic process.

The molecular mechanism by which AEG-1 induces these profound changes is gradually being clarified. AEG-1 is a 582-amino-acid protein with a transmembrane domain and multiple nuclear localization signals.\textsuperscript{1} In cancer cells, AEG-1 is detected in the cytoplasm as well as on the cell membrane and in the nucleus.\textsuperscript{2} Depending upon location, AEG-1 interacts with different protein complexes regulating diverse functions. AEG-1 interacts with nuclear factor kappa light-chain enhancer of activated B cells (NF-κB) and CREB-binding protein (CBP) promoting NF-κB-mediated transcription,\textsuperscript{6} whereas it interacts with YY1, along with CBP, to repress transcription.\textsuperscript{7} In the cytoplasm, AEG-1 is a component of the RNA-induced silencing complex and assists oncomiR-mediated degradation of tumor-suppressor messenger RNAs (mRNAs).\textsuperscript{8} AEG-1 facilitates the translation of specific mRNAs, such as the mRNA for the multidrug resistance gene, multidrug resistance protein 1 (MDR1), which contributes to chemoresistance.\textsuperscript{9} The membrane-located AEG-1 promotes the interaction of cancer cells with lung endothelium, thus augmenting metastasis.\textsuperscript{3} The identification of the diverse interacting partners indicates that AEG-1 may be a scaffold protein mediating the formation of multiprotein complexes in different intracellular compartments.

AEG-1 plays an important role in hepatocarcinogenesis.\textsuperscript{2} AEG-1 mRNA and protein overexpression, as well as amplification of the AEG-1 gene, was detected in a large percentage of hepatocellular carcinoma (HCC) patients.\textsuperscript{2} To better comprehend the role of AEG-1 in hepatocarcinogenesis and to decipher the underlying molecular mechanism(s) in an \textit{in vivo} context, we have generated a transgenic (TG) mouse with hepatocyte-specific expression of AEG-1 (Alb/AEG-1). We document that, compared to wild-type (WT) mice, the hepatocarcinogenic process is significantly amplified in Alb/AEG-1 mice. We unraveled novel aspects of AEG-1, including induction of steatosis, protection from senescence, and activation of coagulation pathways, which contribute to its tumor-promoting functions. This is the first study analyzing AEG-1 function \textit{in vivo}, and the Alb/AEG-1 mouse provides a useful model to further understand the hepatocarcinogenic process and evaluate emerging novel therapies for this invariably fatal disease.

Materials and Methods

\textbf{Generation of Alb/AEG-1 Mouse and Induction of Chemical Carcinogenesis.} The Alb/AEG-1 mouse was generated by directing the expression of human AEG-1 under an upstream enhancer region (−10400 to −8500) fused to the 335-base-pair core region of mouse albumin (ALB) promoter.\textsuperscript{10} Microinjection and manipulation procedures were performed according to standard procedures in the Virginia Commonwealth University Massey Cancer Center Transgenic/Knockout Mouse Facility (Richmond, VA). For induction of chemical carcinogenesis, a single intraperitoneal (IP) injection of 10 μg/g body weight of N-
nitrosodiethylamine (DEN) was given at 14 days of age to male WT and Alb/AEG-1 mice.11

**Cells and Culture Condition.** Primary mouse hepatocytes were isolated from WT and Alb/AEG-1 mice, as previously described.12 Primary human hepatocytes were obtained from the Liver Tissue Cell Distribution System (National Institutes of Health contract #N01-DK-7-0004/HHSN267200700004C) and were cultured in hepatocyte culture medium containing the supplements (Lonza, Walkersville, MD). Human umbilical vein endothelial cells (HUVECs) were obtained from Lonza and were cultured according to the provided protocol.

**Polysome Purification, Total RNA Extraction, Real-Time Polymerase Chain Reaction, and Microarray Assay.** Purification of polysomal fractions from WT and Alb/AEG-1 hepatocytes was performed as previously described.9 Total RNA was extracted from each polysomal fraction and from WT and Alb/ AEG-1 livers using the QIAGEN miRNAeasy Mini Kit (QIAGEN, Hilden, Germany). Real-time polymerase chain reaction (PCR) was performed using an ABI Viia7 fast real-time PCR system and Taqman gene-expression assays according to the manufacturer's protocol (Applied Biosystems, Foster City, CA). An Affymetrix oligonucleotide microarray (GeneChip Mouse Genome 430A 2.0 Array representing approximately 14,000 well-characterized mouse genes; Affymetrix, Santa Clara, CA) analysis was performed to compare gene expression between DEN-treated WT and Alb/ AEG-1 liver samples, as previously described.2

**Mass Spectrometric Analysis of Conditioned Media.** Conditioned media (CM) from WT and Alb/ AEG-1 hepatocytes were collected 1 day after isolation and subjected to mass spectrometric (MS) analysis, as previously described.8 Peptide samples were fractionated by reverse-phase liquid chromatography (LC) and were analyzed by a high-performance LC-MS/MS using an LTQ Orbitrap XL mass spectrometer (Thermo Electron; Thermo Fisher Scientific Inc., Waltham, MA) and utilizing a label-free approach. Two independent replicate MS analyses were carried out per sample.

**Statistical Analysis.** Data are represented as the mean ± standard error of mean (SEM) and were analyzed for statistical significance using one-way analysis of variance, followed by Newman-Keuls’ test as a post-hoc test. A P value of <0.05 was considered as significant.

**Results**

**Generation and Characterization of Alb/AEG-1 Mice.** We have created a TG mouse in a B6/CBA background with hepatocyte-specific expression of human AEG-1 by using the mouse ALB promoter/enhancer element to drive AEG-1 expression. This particular strain of mouse was chosen because it is very sensitive to hepatocarcinogenesis induced by DEN.11 The human AEG-1 has a C-terminal HA-tag. The expression of AEG-1 in the liver of Alb/AEG-1 mice was confirmed by western blotting analysis using anti-HA antibody (Ab) (Fig. 1A). Two founder lines were characterized, initially revealing no significant differences. We therefore pursued further characterization employing one founder line.

**Alb/AEG-1 Mice Develop HCC.** Male WT and Alb/AEG-1 litters were given a single IP injection of DEN (10 μg/g) at 14 days of age and were monitored every 4 weeks, starting at 20 weeks. At 28 weeks of age, only 2 of 11 WT animals showed a few very small nodules in the liver, whereas all of the 17 Alb/ AEG-1 mice livers harbored numerous nodules of different sizes (arrows in Fig. 1B,C). There was a significant increase in liver-to-body-weight ratio in Alb/ AEG-1 mice, when compared to that in WT (Fig. 1D). Histological analysis of the livers of WT mice showed a few dysplastic, hyperchromatic nuclei (arrow in Fig. 2A), indicating that, with time, HCC would eventually develop. In Alb/AEG-1 mice, a marked increase in dysplastic, hyperchromatic nuclei was observed both in the nodules as well as in the adjacent healthy liver (arrows in Fig. 2B,C). The most striking feature was observed in the hepatic nodules of Alb/ AEG-1 mice, showing profound steatotic phenotypes with large lipid droplets in the hepatocytes (Fig. 2C). A moderate level of steatosis was also observed in the adjacent healthy liver in Alb/AEG-1 mice. There was a significant increase in hepatic enzymes in the sera of Alb/AEG-1 mice versus the sera of WT mice (Supporting Fig. 1). At 32 weeks of age, the WT mice developed hepatic nodules; however, the nodules that developed in Alb/AEG-1 mice were markedly larger (Supporting Fig. 2). These findings indicate that AEG-1 significantly accelerated the hepatocarcinogenic process in DEN-treated animals. The WT and Alb/AEG-1 mice were followed for 1 year without any DEN treatment. Although AEG-1-induced steatosis was profoundly evident, overt nodular HCC did not develop at this time point.

**Modulation of Global Gene Expression in Alb/ AEG-1 Mice.** To define AEG-1-induced gene expression changes potentially leading to an oncogenic phenotype, we compared DEN-treated WT and Alb/ AEG-1 livers by performing oligonucleotide microarray experiments using biological triplicates in each animal group. We used samples at 28 weeks, because this
is the earliest time point at which nodules are observed in Alb/AEG-1 mice. Using a 2-fold cutoff and a \( P \) value of \(<0.05\), we identified 25 AEG-1-regulated genes that might contribute to AEG-1 function (Supporting Table 1). A supervised gene-cluster analysis is shown in Supporting Fig. 3. These genes include the following: HCC marker alpha-fetoprotein; invasion- and metastasis-associated genes tetraspanin 8 and lipocalin 2; several genes associated with fat metabolism, such as stearoyl coenzyme A (CoA) desaturase (Scd)2, lipoprotein lipase, apolipoprotein A-IV, and apolipoprotein C-II; and genes regulating angiogenesis, such as trefoil factor 3 (TFF3) and mesenchyme homeobox 2. mRNA and protein expression levels in WT and Alb/AEG-1 mice were validated by real-time PCR and IHC, respectively, using 5 animals per group (Supporting Fig. 4). A significant increase in CD31, a marker for microvessels, was observed in Alb/AEG-1 mice, when compared to WT mice, supporting proangiogenic properties of AEG-1 (Supporting Fig. 4).

**AEG-1 Protects From Chemotherapeutics and Growth Factor Deprivation.** To understand what properties of AEG-1 promote the hepatocarcinogenic process, we isolated and characterized hepatocytes from WT and Alb/AEG-1 mice. The overexpression of AEG-1 was confirmed in hepatocytes by western blotting analysis using both anti-AEG-1 and anti-HA Abs (Supporting Fig. 5). One profound phenotype conferred by AEG-1 is chemoresistance.\(^{3,9,13}\) Indeed, Alb/AEG-1 hepatocytes demonstrated marked resistance to doxorubicin (DOX) and 5-fluorouracil (5-FU) treatment, when compared to their WT littermates (Fig. 3A,B). Primary mouse hepatocytes, cultured in the presence of growth factors, do not divide and show decreasing viability after \( \sim 4 \) days as they enter senescence. The viability of Alb/AEG-1 hepatocytes in complete growth media was significantly higher than that of WT hepatocytes, as monitored by standard tetrazolium (MTT) assay over a 7-day period (Fig. 3C). Upon removal of growth factors, the WT hepatocytes...
started losing viability within 1 day, and by 3 days, more than 50% of the cells were dead (Fig. 3C). In contrast, Alb/AEG-1 hepatocytes were significantly resistant to the removal of growth factors, and even after 7 days in basal media, cell viability was only reduced by 20% (Fig. 3C). These observations indicate that AEG-1 might autonomously activate growth-factor–induced signaling and might inhibit pathways mediating senescence. Indeed, Alb/AEG-1 hepatocytes exhibited higher levels of activated (i.e., phosphorylated) extracellular signal-related kinase (ERK), Akt, and p38 mitogen-activated protein kinase (MAPK) as well as antiapoptotic proteins B-cell lymphoma 2 and myeloid cell leukemia-1, but not B-cell lymphoma-extra large, when compared to WT hepatocytes (Fig. 3D).

**AEG-1 Inhibits Senescence.** WT and Alb/AEG-1 hepatocytes were cultured for 7 days, and senescence was monitored by senescence-associated β-galactosidase (SA-β-gal) assays. At day 7, WT hepatocytes became large and vacuolated and ~55% cells were positive for SA-β-gal, whereas only 3% of Alb/AEG-1 hepatocytes stained positive for SA-β-gal (Fig. 4A). Similarly, the number of senescence-associated heterochromatin foci (SAHF) was ~4 times more per nucleus in WT hepatocytes, when compared to Alb/AEG-1 hepatocytes (Fig. 4B). Senescence might be induced by activation of the Rb/p16 pathway or by activation of a DNA damage-response pathway, leading to activation of p53 and p21. We did not observe any change in activation of Rb (data not shown). However, in WT hepatocytes at day 7, there was significant activation of ataxia telangiectasia mutated (ATM) and ATM and Rad3-related as well as their downstream kinases, CHK1 and CHK2, leading to p53 phosphorylation and increase in p53 and p21 levels (Fig. 4C). In Alb/AEG-1 hepatocytes, there was a marked dampening of the activation of DNA damage response at day 7, indicating that AEG-1 significantly protects from a DNA damage response, thereby nullifying the anticancer process of senescence. To investigate the mechanism of DNA damage response, we measured reactive oxygen species (ROS) levels in WT and Alb/AEG-1 hepatocytes. During the initial period of culture, such as at day 1, there was a significant increase in total ROS level in WT hepatocytes, when compared to that in Alb/AEG-1 hepatocytes (Fig. 4D). At day 7, when WT hepatocytes had become metabolically inactive as a result of senescence, ROS level decreased significantly by ~90%, whereas basal ROS level was higher in Alb/AEG-1 hepatocytes, demonstrating ~75% decrease, indicating more metabolically active cells. The protection from senescence by AEG-1 was also substantiated in primary human hepatocytes (Supporting Fig. 6).

**AEG-1 Profoundly Augments Angiogenesis.** We next investigated the effect of AEG-1 on angiogenesis, another hallmark of cancer. HUVECs were treated for 2 days with CM collected from WT and Alb/AEG-1 hepatocytes. The addition of CM from WT hepatocytes to HUVECs cultured in basal media failed to induce capillary-like structures, whereas CM from Alb/AEG-1 hepatocytes
AEG-1 hepatocytes induced differentiation (Fig. 5A). The proangiogenic property of AEG-1 was further characterized in 9-day-old chick embryos by treating chicken chorioallantoic membrane (CAM) with CM from WT and Alb/AEG-1 hepatocytes. CM from Alb/AEG-1 hepatocytes induced a marked angiogenic response, whereas CM from WT hepatocytes failed to do so (Fig. 5B). To identify the AEG-1-induced proangiogenic secreted factors, we analyzed the CM from WT and Alb/AEG-1 hepatocytes by MS. Interestingly, we identified up-regulation of several components of the coagulation pathway, including fibrinogen α and β chains, factor XII (FXII), plasminogen, and prothrombin, that are known to play significant roles in cancer angiogenesis, metastasis, and invasion (Supporting Table 2). Overexpression of fibrinogen and FXII in the CM of Alb/AEG-1 hepatocytes was confirmed by western blotting analysis (Fig. 5C).

We focused on TFF3 and FXII on their ability to regulate AEG-1-induced angiogenesis. TFF3 and FXII were knocked down by short interfering RNA (siRNA) in WT and Alb/AEG-1 hepatocytes (Supporting Fig. 7), and the CM were subjected to HUVEC differentiation and CAM assays. Although control siRNA did not affect angiogenesis induced by CM from Alb/AEG-1 hepatocytes, inhibition of either TFF3 or FXII resulted in marked inhibition of angiogenesis (Fig. 6A,B). It should be noted that although inhibition of either TFF3 or FXII abrogated sprouting of small vessels in a similar magnitude, knocking down FXII exhibited more-pronounced inhibition in the growth of larger blood vessels, suggesting a pivotal role of FXII in mediating AEG-1-induced angiogenesis.

FXII cross-talks with epidermal growth factor receptor (EGFR), activating MAPK and Akt signaling to promote proliferation and differentiation of endothelial cells (ECs). We treated HUVECs with CM from Alb/AEG-1 hepatocytes transfected with either control siRNA or FXII siRNA. Although CM from control

AEG-1 hepatocytes induced differentiation (Fig. 5A).

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siRNA-treated HUVECs maintained activation of EGFR, Akt, ERK, and p38 MAPK, the absence of FXII in the CM form FXII knock-down cells significantly abrogated the activation of EGFR, Akt, ERK, and p38 MAPK in HUVECs (Fig. 6C). These findings further support that FXII plays an important role in AEG-1-induced proliferation and differentiation of ECs.

**AEG-1 Up-regulates FXII Translation.** We analyzed FXII mRNA level in WT and Alb/AEG-1 hepatocytes and observed only modest changes (data not shown), indicating that AEG-1 may preferentially increase FXII at the protein level. In WT hepatocytes, AEG-1 is expressed at low levels and is predominantly localized in the nucleus (Fig. 7A). In contrast, in Alb/AEG-1 hepatocytes, AEG-1 is almost exclusively contained in the cytoplasm (Fig. 7A). We hypothesized that cytoplasmic AEG-1 might augment the translation of FXII mRNA by facilitating its association with polysomes. Polysomal fractions were collected from WT and Alb/AEG-1 hepatocytes, RNA was extracted from each fraction, and an equal amount of RNA from each fraction was subjected to complementary DNA synthesis and Taqman real-time PCR for FXII. The mean cycle threshold (Ct) value for FXII amplification was significantly lower in Alb/AEG-1 hepatocytes, compared to WT hepatocytes, indicating that AEG-1...
preferentially helps FXII mRNA associate with poly-

Discussion

The oncogenic properties of AEG-1 have been val-

Plasma FXII analysis thus might be a potential bio-

One novel aspect of AEG-1 function is the induc-

regulation of coagulation factors. AEG-1 induces

In addition to increased polysomal association of FXII

mRNAs to the polysome. Indeed, ribosomal proteins

inhibiting FXII or TFF3 results in a marked inhibition of

AEG-1-induced angiogenesis. Interestingly, both FXII

pathway, all of which are known mediators of tumor
growth, metastasis, and angiogenesis, and this particu-

The question is, does AEG-1 also regulate FXII under normal condi-
tions? Will AEG-1 knock-out (KO) mice suffer from clotting deficiencies? The answer is most likely not. In
primary mouse hepatocytes, AEG-1 is predominantly
localized in the nucleus or nucleolus. However, when
overexpressed, AEG-1 is most abundantly detected in
the cytoplasm, a phenomenon also observed in human
HCC patients as well as human HCC cells stably
overexpressing AEG-1.2,22 In prostate cancer cells,
AEG-1 is monoubiquitinated, resulting in its stabiliza-
tion and cytoplasmic sequestration.23 A similar mecha-
nism might also be applicable to HCC cells as well as
in Alb/AEG-1 hepatocytes, and the monoubiquitina-
tion of overexpressed AEG-1 was confirmed (Support-
ing Fig. 9). The promiscuous accumulation of AEG-1
in the cytoplasm might facilitate an interaction with
the translational machinery and loading of selective
mRNAs to the polysome. Indeed, ribosomal proteins
as well as eukaryotic translation initiation factors were
identified as potential AEG-1-interacting proteins,
indicating a potential direct role of AEG-1 in regulat-
ing translation.8 It is intriguing that AEG-1 facilitates
the translation of multiple members of the coagulation
pathway, all of which are known mediators of tumor
growth, metastasis, and angiogenesis, and this particu-
lar aspect of AEG-1 function might play a pivotal role
in promoting tumor progression and metastasis.

Our studies unravel the striking observation that
AEG-1 provides strong protection from senescence,
a phenomenon that may not be explicit in immortal
healthy cells. AEG-1 provided a strong inhibition to the
DNA damage response induced in hepatocytes as they
age and protected them from senescence. We observed
that during the initial culture period, the endogenous
ROS level was ~30% lower in Alb/AEG-1 hepatocytes
versus WT hepatocytes. This initial level of increased
endogenous ROS in WT hepatocytes might be suffi-
tient to trigger the DNA damage response resulting in
senescence. Senescence is a potential anticancer mecha-
nism,20 and by blocking senescence, AEG-1 may further
promote the carcinogenic process.

We demonstrate an intriguing aspect of AEG-1
when it is overexpressed, resulting in translational up-

One novel aspect of AEG-1 function is the induc-
tion of steatosis. Nonalcoholic fatty liver disease
(NAFLD) is one of the precursors leading to

We analyzed the expression levels of mature miR-181a
in WT and Alb/AEG-1 hepatocytes and did not
observe any difference (Supporting Fig. 8). Thus,
miRNA-mediated regulation might not be a major
mechanism of FXII induction by AEG-1.
nnonalcoholic steatohepatitis and HCC. It will be interesting to check whether AEG-1 is also overexpressed in NAFLD patients, thus contributing to eventual hepatocarcinogenesis. Apart from significant increases in the expression of some components of fatty acid metabolism, our gene-expression network analysis did not identify the modulation of any major adipogenic or lipogenic pathway, such as the peroxisome proliferator-activated receptor gamma, liver X receptor, or pregnane X receptor pathways. This observation argues that rather than affecting a network, AEG-1 overexpression might lead to promiscuous increases in distinct regulators of fat metabolism resulting in steatosis. The significant increase in SCD2 expression by AEG-1 alone might contribute to steatosis. Induction in SCD2 has also been observed in the transforming growth factor alpha/c-myc TG mouse model of HCC. SCDs are crucial lipogenic enzymes for monounsaturated fatty acid biosynthesis. SCD1 expression is induced after weaning in mouse liver, whereas SCD2 expression is detected in livers of mouse embryos and neonates. There is a significant reduction in liver and plasma triglycerides in neonatal SCD2 KO mice. The increased SCD2 expression by AEG-1 suggests a shift toward embryonic gene-expression pattern, another hallmark of cancer. Crossing SCD2 KO mice with Alb/AEG-1 mice might provide insight into the importance of SCD2 in mediating the AEG-1-induced steatotic phenotype. Additionally, similarly to FXII, AEG-1 might also lead to changes in specific adipogenic or lipogenic factors only at the protein level. Indeed, our preliminary studies reveal the overexpression of fatty acid synthase and acetyl CoA carboxylase, two important enzymes of fatty acid synthesis, in livers of Alb/AEG-1 mice (Supporting Fig. 10). A detailed study analyzing the molecular mechanism of AEG-1-induced steatosis is currently under way.

In summary, the Alb/AEG-1 mouse uncovers several novel aspects of AEG-1 function that might not be possible using in vitro models and nude mice xenograft studies. Characterization of this model facilitates the identification of potential biomarkers that might be further validated in HCC patient samples. This mouse model might also be valuable in evaluating novel therapeutic approaches targeted toward NAFLD and HCC.

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