Published in final edited form as:

Adv Cancer Res. 2013 ; 120: 135–157. doi:10.1016/B978-0-12-401676-7.00005-X.

Drug resistance mediated by AEG-1/MTDH/LYRIC

Xiangbing Meng^{1,2}, Kristina W Thiel¹, and Kimberly K Leslie^{1,2}

¹Department of Obstetrics and Gynecology, The University of Iowa, Iowa City, IA 52242

²Holden Comprehensive Cancer Center, The University of Iowa, Iowa City, IA 52242

Abstract

AEG-1/MTDH/LYRIC has been shown to promote cancer progression and development. Overexpression of AEG-1/MTDH/LYRIC correlates with angiogenesis, metastasis and chemoresistance to various chemotherapy agents in cancer cells originating from a variety of tissues. In this review article, we focus on the role of AEG-1/MTDH/LYRIC in drug resistance. Mechanistic studies have shown that AEG-1/MTDH/LYRIC is involved in classical oncogenic pathways including Ha-Ras, myc, NFκB and PI3K/Akt. AEG-1/MTDH/LYRIC also promotes protective autophagy by activating AMP kinase and autophagy-related gene 5. Another reported mechanism by which AEG-1/MTDH/LYRIC regulates drug resistance is by increasing loading of multidrug resistance gene (MDR) 1 mRNA to the polysome, thereby facilitating MDR1 protein translation. More recently, a novel function for AEG-1/MTDH/LYRIC as an RNA binding protein was elucidated, which has the potential to impact expression of drug sensitivity or resistance genes. Finally, AEG-1/MTDH/LYRIC acts in microRNA-directed gene silencing via an interaction with staphylococcal nuclease and tudor domain containing 1 (SND1), a component of the RNA-induced silencing complex. Altered microRNA expression and activity induced by AEG-1/MTDH/LYRIC represents an additional way that AEG-1/MTDH/LYRIC may cause drug resistance in cancer. The multiple functions of AEG-1/MTDH/LYRIC in drug resistance highlight that it is a viable target as an anti-cancer agent for a wide variety of cancers.

Keywords

AEG-1/MTDH/LYRIC; SND1; NFκB; miR-375; chemoresistance

I. Introduction: AEG-1/MTDH/LYRIC, a gene involved in cancer metastasis and drug resistance

Novel treatment strategies are necessary to improve outcomes for cancer patients with drug resistance and metastasis, which combined are responsible for greater than 90% of cancer-related deaths (Ahmad et al.). Drug resistance can be classified into two categories: intrinsic resistance and acquired resistance. Intrinsic resistance refers to resistance mechanism(s) that are present in the tumor prior to treatment, whereas acquired resistance develops during the course of treatment. Mechanisms of drug resistance include genetic mutations, epigenetic alterations, induced expression of drug targets, overexpression of drug resistance genes (i.e., genes involved in drug efflux), increased repair of DNA damage, reduced apoptosis, drug-induced mutations, altered metabolism of the drug, and expression of microRNAs. Given this wide range of causes of drug resistance, future treatment strategies must be tailored to the specific mediator of drug resistance. In this review, we discuss one such specific target,

To whom correspondence should be addressed: Xiangbing Meng, PhD, Department of Obstetrics and Gynecology, The University of Iowa, 200 Hawkins Drive, Iowa City, IA, 52242 USA, xiangbing-meng@uiowa.edu, Phone: (319)335-8212, Fax: (319)356-3901.

AEG-1/MTDH/LYRIC, and the current knowledge regarding its contribution to drug resistance.

AEG-1/MTDH/LYRIC was recently identified as an oncogene that functions in both drug resistance and metastasis (Nestal de Moraes et al., 2012). Originally discovered as an HIV-1-inducible gene in fetal astrocytes (Kang et al., 2005), AEG-1/MTDH/LYRIC overexpression has now been documented in many cancers, including breast, prostate, esophageal, gastric, renal, colorectal, lung, hepatocellular, ovarian and endometrial cancers, neuroblastoma, glioma and sarcoma (Bhutia et al., 2010; Hu et al., 2009b; Li et al., 2012a; Meng et al., 2011a; Qian et al., 2011; Srivastava et al., 2012a; Srivastava et al., 2012b; Thirkettle et al., 2009b; Wang and Yang, 2011; Ying et al., 2011; Yoo et al., 2010; Yoo et al., 2011a; Yoo et al., 2009b). AEG-1/MTDH/LYRIC is located at chromosome 8q22, a region that is frequently amplified in various cancers, including breast and hepatocellular carcinoma (HCC) (Bergamaschi et al., 2006; Hu et al., 2009a; Poon et al., 2006). Overexpression of AEG-1/MTDH/LYRIC has been observed in >90% of neuroblastomas and HCC (Lee et al., 2009). AEG-1/MTDH/LYRIC was identified as a metastasis gene in a phage expression library screen from metastatic breast carcinoma, which was designed to identify proteins that bind to the vasculature of the lung (Brown and Ruoslahti, 2004). Further study revealed AEG-1/MTDH/LYRIC overexpression to be independently associated with a poor disease-free survival rate and a poor distant metastasis-free survival rate in breast cancer (Tokunaga et al., 2012).

The first evidence of a role for AEG-1/MTDH/LYRIC in chemoresistance came from pharmacogenomic analysis of the NCI-60 panel of cancer cells. In this study, the AEG-1/MTDH/LYRIC DNA copy number was found to reversely correlate with sensitivity to chemotherapeutic agents (Hu et al., 2009a). We now understand that overexpression of AEG-1/MTDH/LYRIC confers broad drug resistance to chemotherapeutic agents, including 5-fluorouracil (5-FU), doxorubicin, paclitaxel, and cisplatin, as well as to targeted therapies (Table 1). In this review, we will discuss the known mechanisms of AEG-1/MTDH/LYRIC-mediated drug resistance to date as well as the contribution of other genes near AEG-1/MTDH/LYRIC on chromosome 8q22 that are also amplified in tumors.

II. Mechanisms of AEG-1/MTDH/LYRIC-mediated drug resistance

A. Inhibition of apoptosis

Given the high rate of AEG-1/MTDH/LYRIC overexpression in many types of cancer, along with its association with resistance to multiple types of therapy, many studies have explored how AEG-1/MTDH/LYRIC mediates these detrimental effects. One potential mechanism is through activation of pro-survival pathways. For example, overexpression of AEG-1/MTDH/LYRIC increases cell survival in response to serum deprivation (Kikuno et al., 2007; Lee et al., 2008). AEG-1/MTDH/LYRIC-mediated activation of PI3K/Akt pro-survival signaling, and down-regulation of Bad, p21, p27 and FOXO3a may be involved in this process (Kikuno et al., 2007; Lee et al., 2008). In addition, knockdown of AEG-1/MTDH/LYRIC increases expression and activation of FOXO3a by promoting its translocation to nucleus via an AEG-1/MTDH/LYRIC/ERK1/2 pathway rather than an AEG-1/MTDH/LYRIC/Akt pathway (Wilson et al., 2011). Thus, AEG-1/MTDH/LYRIC accomplishes escape from apoptosis through multiple mechanisms, all of which may contribute to its role in chemoresistance.

In addition to activating pro-survival pathways, AEG-1/MTDH/LYRIC overexpression also mediates resistance to therapies that mediate apoptosis, whether it is chemotherapy or targeted therapy such as death receptor ligand TRAIL (tumor necrosis factor-related apoptosis-inducing ligand), angiogenesis inhibitor BIBF1120, or MAPK/ERK kinase

(MEK) inhibitor AZD6244 (Kong et al., 2012). TRAIL is the ligand that activates the death receptor-mediated extrinsic apoptosis pathway (Holoch and Griffith, 2009). TRAIL is important as an anti-cancer therapy because it induces cancer-specific apoptosis without impairing normal cells (Walczak et al., 1999). TRAIL-mediated apoptosis can be enhanced by inhibition of histone deacetylase (HDAC) activity in a mechanism that includes increased expression of death receptors (Meng et al., 2011b). In a recent study, we found that knockdown of AEG-1/MTDH/LYRIC using a specific shRNA can further increase endometrial cancer cell death induced by TRAIL and HDAC inhibitor combination treatment via inhibiting anti-apoptotic gene X-linked inhibitor of apoptosis protein (XIAP) and increasing activation of caspases 3 and 8 (Meng et al., 2011b). AEG-1/MTDH/LYRIC also contributes to resistance to BIBF1120, an angiogenesis inhibitor that targets multiple pro-angiogenic receptors including platelet-derived growth factor receptor (PDGFR), vascular endothelial growth factor receptor (VEGFR), and fibroblast growth factor receptor (FGFR). In endometrial cancer cells that express these angiogenic receptors, silencing of AEG-1/MTDH/LYRIC sensitizes cancer cells to BIBF1120 (Meng et al., 2012). Finally, AEG-1/MTDH/LYRIC is associated with resistance to AZD6244, an ATP-noncompetitive inhibitor of MAPK/MEK1/2 that has been used in clinical trials (Kong et al., 2012). Resistance to AZD6244 has been reversed by depleting AEG-1/MTDH/LYRIC in breast cancer cell lines (Kong et al., 2012). These examples highlight the potential to restore sensitivity to multiple distinct types of therapy by inhibiting AEG-1/MTDH/LYRIC function.

B. Role in protective autophagy

Autophagy is a lysosomal degradation pathway that participates in the degradation of cytosolic proteins, macromolecules, organelles, and protein aggregates to maintain cellular homeostasis (Lum et al., 2005). As such, activation of autophagy may function in a tumor suppressive capacity by degrading defective cells. However, the autophagy process may also be co-opted by cancer cells to survive during periods of stress, such as exposure to chemotherapy. In immortalized primary human fetal astrocyte cells, AEG-1/MTDH/LYRIC induces autophagy by decreasing the ATP/AMP ratio, which in turn activates AMP kinase (AMPK) and a non-canonical autophagy pathway (Bhutia et al., 2010). Autophagy can also be surveyed by monitoring accumulation of LC3-II, an autophagy marker. Silencing AEG-1/MTDH/LYRIC in multiple cancer cell lines (TG98, HeLa, MDA-MB-231, HO-1, and MIA PaCa 2 cells) restores chemosensitization by decreasing accumulation of LC3-II, indicative of a decrease in activation of protective autophagy (Bhutia et al., 2010). Collectively, these data support a role for AEG-1/MTDH/LYRIC in induction of protective autophagy following cellular stress through multiple possible mechanisms.

C. Activation of transcription factor NFκB

AEG-1/MTDH/LYRIC is expressed in multiple cellular compartments, including the nucleus. Some of the first studies of AEG-1/MTDH/LYRIC function identified a role as a transcription co-factor based on its interaction with nuclear factor κB (NFκB) p65 subunit (Emdad et al., 2006). Overexpression of AEG-1/MTDH/LYRIC in HeLa cells results in induction of several NFκB downstream genes, including as intercellular adhesion molecule (ICAM)-2, ICAM-3, E-, L-, and P-selectin, interleukin (IL)-6, IL-8, Toll-like receptor (TLR)-4, TLR-5, matrix metalloproteinase-9 (MMP9), c-Jun and c-Fos (Emdad et al., 2006; Kikuno et al., 2007). The region of AEG-1/MTDH/LYRIC that directly binds p65 was mapped to residues 101–201 and is termed the p65-interaction domain. AEG-1/MTDH/LYRIC binding to p65 increases its nuclear translocation and thus transcriptional activity. In addition to modulating NFκB localization, AEG-1/MTDH/LYRIC has also been shown to facilitate the interaction between NFκB and its co-factor cAMP response element-binding protein (CREB) binding protein (CBP) in glioma cells (Sarkar et al., 2008). In contrast to

direct binding to p65, the N-terminal 71 residues of AEG-1/MTDH/LYRIC are required for the interaction with CBP and corresponding NF κ B activation (Sarkar et al., 2008). Alternatively, AEG-1/MTDH/LYRIC may indirectly activate NF κ B-mediated transcriptional activation in a pathway that includes PI3K/Akt. Specifically, PI3K/Akt promotes activation of I κ B kinase (IKK) (Nohata et al.), which in turn phosphorylates the NF κ B inhibitor I κ B. I κ B phosphorylation results in its destabilization, thus relieving NF κ B inhibition.

By modulating NF κ B activation state, AEG-1/MTDH/LYRIC regulates NF κ B transcription of target genes. However, AEG-1/MTDH/LYRIC may also play a role in NF κ B transcription of microRNAs, thus expanding the milieu of genes that are altered by AEG-1/MTDH/LYRIC. NF κ B has been reported to regulate the expression of select microRNAs, including miR-21 and miR-221 (Galardi et al., 2011). miR-21 targets the tumor suppressor gene PTEN and pro-apoptotic gene programmed cell death 4 (PDCD4). Overexpressing staphylococcal nuclease and tudor domain containing 1 (SND1), a AEG-1/MTDH/LYRIC interacting protein, is also associated with an increase in NF κ B-mediated miR-221 expression (Santhekadur et al., 2012), providing further evidence for an intimate link between AEG-1/MTDH/LYRIC and NF κ B in the mechanism of cell survival.

D. Regulation of translation

In addition to nuclear expression, AEG-1/MTDH/LYRIC is highly expressed in the cytoplasm and endoplasmic reticulum (ER), indicating that AEG-1/MTDH/LYRIC may alter gene expression by regulating translation. Indeed, AEG-1/MTDH/LYRIC has been reported to interact with ribosomal proteins and translation factors to control protein translation, with potential implications in drug resistance. Other studies have provided evidence that suggest a global role for AEG-1/MTDH/LYRIC in translation. Specifically, AEG-1/MTDH/LYRIC has been shown to increase phosphorylation of eIF4G, which is required for the recruitment of eIF4G to the 5'-cap of mRNA and translation initiation (Yoo et al., 2010a).

i. AEG-1/MTDH/LYRIC promotes mRNA loading in the polysome—Resistance to the anthracycline doxorubicin is a very common event in anti-cancer treatment. AEG-1/MTDH/LYRIC has been implicated in altering the uptake or retention of chemotherapy drugs in some cancer cells (Yoo et al., 2010). The most frequent mechanism of chemoresistance is increased expression of multidrug resistance gene 1 (MDR1), a member of the ATP-binding cassette (ABC) transporter family that mediates drug efflux (Chen and Tiwari, 2011). AEG-1/MTDH/LYRIC has been found to increase MDR1 protein translation by increasing MDR1 mRNA loading in polysomes without altering MDR1 mRNA levels (Yoo et al., 2010). This increase in MDR1 protein expression promotes drug resistance in cancer cells by facilitating export of chemotherapy agents. Treatment of cells with a PI3K inhibitor inhibits AEG-1/MTDH/LYRIC-mediated loading of MDR1 to polysomes (Yoo et al., 2010), implicating PI3K/Akt signaling in the mechanism. Perhaps more importantly, these data suggest that PI3K inhibitors, which are currently in the clinical pipeline, may represent a strategy to block AEG-1/MTDH/LYRIC function in cancer. Other mRNAs that encode ABC transporters interact with AEG-1/MTDH/LYRIC (Meng et al., 2012), though no studies have provided a link between this association and drug resistance. Interestingly, AEG-1/MTDH/LYRIC also inhibits ubiquitination of MDR1 and subsequent proteasome-mediated degradation by an unknown mechanism (Yoo et al., 2010), suggestive of alternate mechanisms by which AEG-1/MTDH/LYRIC regulates MDR1 to achieve drug resistance.

In addition to regulating MDR1 mRNA association with the polysome, AEG-1/MTDH/LYRIC increases factor XII (FXII) protein translation by increasing its loading to the

polysome (Srivastava et al., 2012a). FXII is a serum glycoprotein that participates in the initiation of blood coagulation and angiogenesis, indicating that AEG-1/MTDH/LYRIC, through regulation of FXII, may affect drug bioavailability.

ii. AEG-1/MTDH/LYRIC acts as a scaffold protein to form multi-protein and RNA complexes—To date, several intracellular AEG-1/MTDH/LYRIC binding partners have been identified, and some of these interactions explain in part the aggressive phenotype of AEG-1/MTDH/LYRIC-overexpressing cancers. Relevant to the role of AEG-1/MTDH/LYRIC in protein translation, mass spectrometry revealed an association of AEG-1/MTDH/LYRIC with multiple RNA binding proteins, ribosomal proteins and translation regulatory proteins (Meng et al., 2012; Yoo et al., 2011c). Some of these interactions are disrupted by nuclease (i.e., SND1, NPM and B23) (Meng et al., 2012), leading to the hypothesis that AEG-1/MTDH/LYRIC binds RNA. Consistent with this notion, putative RNA binding regions have been identified in AEG-1/MTDH/LYRIC. Moreover, RNA-binding protein immunoprecipitation followed by microarray analysis (RIP-chip) revealed that AEG-1/MTDH/LYRIC associates with various mRNA sequences (Meng et al., 2012). The proteins encoded by these mRNAs are implicated in a wide range of cellular processes (Table 2), including DNA repair (FANCC, FANCA, FANCD2, FANCI), epigenetic regulation (KDM6A, WHSC1L1), signal transduction (PDCD11, OSMR), and RNA metabolism (DDX6, DDX60, DDX21). While inhibition of PI3K signaling alters the association of AEG-1/MTDH/LYRIC with mRNAs (Meng et al., 2012), the effect of AEG-1/MTDH/LYRIC on translation and stability of these AEG-1/MTDH/LYRIC-associated mRNAs requires further investigation, as well as determining whether AEG-1/MTDH/LYRIC mRNA binding plays a role in drug resistance.

E. Inhibition of stress granule formation

In response to cellular stress such as heat shock, the stalled translation complex forms stress granules, which can be detected by staining with Ras-GAP SH3 domain binding protein (G3BP) (Unsworth et al., 2010). While cytosolic mRNAs aggregate into stress granules, ER-bound transcripts escape sequestration in stress granules. SND1, the AEG-1/MTDH/LYRIC interacting protein, was found in stress granules (Weissbach and Scadden, 2012), though AEG-1/MTDH/LYRIC does not accumulate there. Interestingly, MDR1 is one of the genes that escape from stress granules, thus allowing drug resistance (Unsworth et al., 2010). As mentioned above, AEG-1/MTDH/LYRIC can increase MDR1 translation and inhibit MDR1 degradation, which is consistent with the absence of AEG-1/MTDH/LYRIC in stress granules. We recently reported that AEG-1/MTDH/LYRIC depletion increases stress granule formation in response to heat shock stimulation (Meng et al., 2012), indicating negative regulation of stress granules by AEG-1/MTDH/LYRIC through an unknown mechanism. These data suggest that prevention of stress granule formation is one mechanism by which AEG-1/MTDH/LYRIC mediates drug resistance.

F. Regulation of gene silencing

Several lines of evidence have demonstrated that AEG-1/MTDH/LYRIC regulates microRNA expression. Examples mentioned above include regulation of NF κ B transcription of select microRNAs. Below we describe other reported roles for AEG-1/MTDH/LYRIC in post-transcriptional gene silencing.

i. AEG-1/MTDH/LYRIC regulates RNA interference mediated by siRNAs and microRNAs—A growing body of literature demonstrates that a single microRNA can impact hundreds of targets and also that a single target can be affected by multiple microRNAs (Finnegan and Pasquinelli, 2013). Both SND1 and AEG-1/MTDH/LYRIC have been implicated in siRNA- and microRNA-directed gene silencing (Yoo et al., 2011c). In

addition to roles in transcription, splicing, and degradation, SND1 is a component of the RNA-induced silencing complex (Cieply et al.) that interacts with Ago-2 to regulate RNA interference (RNAi). In vitro experiments have demonstrated the critical roles of AEG-1/MTDH/LYRIC and SND1 in optimal activity of RISC to down-regulate tumor suppressor genes by microRNAs. Modulation of AEG-1/MTDH/LYRIC or SND1 expression in HCC cells produces the same effects: overexpression of AEG-1/MTDH/LYRIC or SND1 results in decreased levels of PTEN due to silencing by miR-221 and miR-21, decreased cyclin-dependent kinase inhibitor 1A (CDKN1A, also p21) by miR-106b, decreased sprouty homolog 2 (SPRY2) by miR-21, and decreased transforming growth factor- β receptor II (TGFR2) by miR-93 (Yoo et al., 2011c). AEG-1/MTDH/LYRIC or SND1 knockdown produces the anticipated opposing effects. The interaction of AEG-1/MTDH/LYRIC with SND1 has been observed in several types of cancer (Blanco et al., 2011; Yoo et al., 2011c). Like AEG-1/MTDH/LYRIC, SND1 is recognized as a pro-metastatic gene in breast cancer (Blanco et al 2011). SND1 also acts as a transcription co-factor via interactions with signal transducer and activator of transcription (STAT)6 (Valineva et al., 2005), myb and the serine/threonine kinase PIM1 (Levenson et al., 1998). Thus, while AEG-1/MTDH/LYRIC and SND1 play clear roles in gene silencing, it is unknown whether AEG-1/MTDH/LYRIC also contributes to the transcription co-factor function of SND1.

ii. AEG-1/MTDH/LYRIC causes drug resistance by regulating microRNAs via crosstalk with other pathways—In addition to the above-described regulation of NF κ B transcription of miR-21 and miR-221, other mechanisms have been described by which AEG-1/MTDH/LYRIC alters transcription of particular microRNAs. For example, AEG-1/MTDH/LYRIC is intricately linked with c-myc, a transcription factor that has been associated with altered expression of multiple microRNAs (Chang et al., 2008). Not only does c-myc regulate AEG-1/MTDH/LYRIC transcription (Thirkettle et al., 2009b), but it also induces expression of the miR-17-92 cluster and represses expression of Let-7, and both of these alterations are associated drug resistance (Lu et al., 2011; Totary-Jain et al., 2013). Expression of c-myc is controlled by β -catenin via the M2 isoform of pyruvate kinase (PKM2) (Tamada et al., 2012). PKM2 is a splice isoform of pyruvate kinase that is overexpressed in cancer and plays a critical role in aerobic glycolysis (also known as the Warburg effect) and non-glycolytic transcriptional regulation of multiple genes, including c-myc. In a positive feedback loop, c-myc increases transcription of heterogeneous nuclear ribonucleoprotein (hnRNP), which in turn plays a role in alternative splicing and enhanced PKM2 isoform expression in cancer (Chen et al., 2010). Providing further complexity to this feedback loop, AEG-1/MTDH/LYRIC increases c-myc transcription by either impairing PLZF-mediated transcriptional repression or by activating β -catenin-mediated c-myc transcription (Thirkettle et al., 2009b). Thus, in the setting of AEG-1/MTDH/LYRIC overexpression, the end result of these positive feedback loops may be amplification of the c-myc-mediated effects on microRNA expression and corresponding drug resistance.

In addition to c-myc, AEG-1/MTDH/LYRIC expression has been associated with repression of transcription factors FOXO1 and FOXO3a via activating the PI3K/Akt pathway (Kikuno et al., 2007). Repression of FOXO1 and FOXO3a leads to activation of FoxM1 expression, another transcription factor involved in transcriptional regulation of microRNAs (Wilson et al., 2011). Specifically, FoxM1 transcribes miR-135a, which targets metastasis suppressor 1 (MTSS1) (Liu et al., 2012) and is correlated with drug resistance to paclitaxel (Holleman et al., 2011). Thus, AEG-1/MTDH/LYRIC-mediated activation of downstream FoxM1 results in an increase in miR-135 and the emergence of a resistant phenotype.

iii. AEG-1/MTDH/LYRIC is involved in resistance to endocrine therapies for breast cancer by reducing expression of miR-375—Almost 70% of breast cancer cases are estrogen receptor (ER)- α positive. For these cancers, endocrine therapies of

selective ER modulators (SERMs), such as tamoxifen, are widely applied (Rodriguez Lajusticia et al., 2008). Although these treatments reduce breast cancer mortality, and many ER- α -positive tumors initially respond well, resistance to treatment usually develops. Studies in an *in vitro* model of tamoxifen resistance in breast cancer cells demonstrate that alterations in microRNA expression are associated with the acquisition of tamoxifen resistance (Ward et al., 2012). miR-375 was one of the most significantly downregulated microRNAs in that study. Dysregulated expression of miR-375 has also been detected in lung cancer, squamous cervical cancer, colon cancer and glioma (Bierkens et al., 2013; Chang et al., 2012; Dai et al., 2012; He et al., 2012; Isozaki et al., 2012; Kinoshita et al., 2012; Li et al., 2012b; Nohata et al., 2011; Ward et al., 2012). Interestingly, AEG-1/MTDH/LYRIC is a validated target of miR-375 (Ward et al., 2012). Elevated AEG-1/MTDH/LYRIC levels inversely correlate with miR-375 expression and positively correlate with poorer disease-free survival in tamoxifen-treated breast cancer patients (Ward et al., 2012). Re-expression of miR-375 in tamoxifen-resistant breast cancer cells sensitizes cells to tamoxifen and inhibits their invasive capacity (Ward et al., 2012). Moreover, depletion of AEG-1/MTDH/LYRIC also partly reverses resistance to tamoxifen (Ward et al., 2012).

G. Regulation of tumor microenvironment

In addition to the tumor, cells in the surrounding stroma (i.e., fibroblasts, endothelial or mesothelial cells, adipose tissue-derived stromal cells, and immune cells) may also contribute to chemoresistance. Overexpression of AEG-1/MTDH/LYRIC increases the expression of molecular markers of angiogenesis, including angiopoietin-1, Tie 2, MMP2, and hypoxia-inducible factor 1- α (HIF1- α) (Emdad et al., 2009). To understand the role of AEG-1/MTDH/LYRIC in HCC etiology, a transgenic mouse model with hepatocyte-specific AEG-1/MTDH/LYRIC overexpression was recently generated (Srivastava et al 2012). Not only do these mice develop HCC tumors following challenge with N-nitrosodiethylamine, but conditioned media obtained after culture of isolated AEG-1/MTDH/LYRIC-overexpressing hepatocytes induces angiogenesis as well as an increase in coagulation factor FXII. Increased FXII loading to the polysome, as described earlier, is responsible for changes in FXII expression. A correlation between increased expression of AEG-1/MTDH/LYRIC and VEGF has also been observed in patients with triple negative breast cancer (Li et al., 2011a), though no mechanistic studies have been reported.

III. AEG-1/MTDH/LYRIC downstream genes identified by microarray

In order to achieve a better understanding of how AEG-1/MTDH/LYRIC promotes drug resistance, microarray analysis has been used to explore the downstream genes that are altered by AEG-1/MTDH/LYRIC expression in several cancer types, with a focus on genes that have been previously implicated in resistance. In this section, we describe AEG-1/MTDH/LYRIC-mediated gene regulation in breast, hepatocellular, and endometrial cancers, which all have extremely high expression of AEG-1/MTDH/LYRIC in a majority of tumors.

A. Breast cancer

In breast cancer cells, AEG-1/MTDH/LYRIC knockdown leads to decreased expression of chemoresistance genes aldehyde dehydrogenase 3 family, member A1 (ALDH3A1), MET (the receptor for hepatocyte growth factor), heat shock protein (HSP)90, and heme oxygenase (decycling) 1 (HMOX1) and increased expression of pro-apoptotic genes BCL2/adenovirus E1B 19kDa interacting protein 3 (BNIP3) and TRAIL (Hu et al., 2009a). Simultaneous knockdown of MET and ALDH3A1, an antioxidant enzyme that scavenges free radicals, results in chemosensitization to levels comparable to those observed with AEG-1/MTDH/LYRIC knockdown (Hu et al., 2009a). Constitutively overexpressing of ALDH3A1 or MET partially rescues resistance to paclitaxel, doxorubicin, and 4-

hydroxycyclophosphamide (4-HC) in AEG-1/MTDH/LYRIC knockdown LM2 cells (Hu et al., 2009a).

B. Hepatocellular carcinoma

AEG-1/MTDH/LYRIC is overexpressed in the vast majority (>90%) of HCC tumors. Microarray analysis of AEG-1/MTDH/LYRIC overexpression in HepG3 cells revealed a panel of genes that may also contribute to chemoresistance that were distinct from those identified in breast cancer cells (Yoo et al., 2009b). Some of the most significantly altered genes include those that encode enzymes that metabolize chemotherapeutic agents: dihydropyrimidine dehydrogenase (DPYD), cytochrome P450 2B6 (CYP2B6), and dihydrodiol dehydrogenase (AKR1C2). These genes may contribute to the broad-spectrum role of AEG-1/MTDH/LYRIC in chemoresistance in different cancer types. For example, DPYD expression and activity modulate the efficacy of 5-FU, a common chemotherapeutic agent used to treat HCC (Yoo et al., 2009b).

Consistent with changes in DPYD expression in AEG-1/MTDH/LYRIC-overexpressing cells, it has been shown that overexpression of AEG-1/MTDH/LYRIC increases resistance of HCC cells to 5-FU by induction of DPYD as well as upregulation of thymidylate synthase (TS) by the oncogenic transcription factor late SV40 factor (LSF) (Yoo et al., 2011b). In addition to TS, LSF transcribes several genes associated with aggressive cancers, including osteopontin (OPN), MMP9, MET, and complement factor H (CFH) (Yoo et al., 2011b). A high-throughput screen identified factor quinolinone inhibitor 1 (FQI1) as an LSF DNA binding inhibitor that induces apoptosis and inhibits proliferation of HCC cells (Grant et al., 2012). Because specific inhibitors to target AEG-1/MTDH/LYRIC have not been identified, inhibiting AEG-1/MTDH/LYRIC downstream effector LSF may provide a new strategy to treat HCC. Indeed, LSF may very well represent an “Achilles heel” for HCC given the addiction to LSF for survival (Grant et al., 2012).

The microarray analysis of HCC cells also revealed that mRNA levels of the ABC transporter ABCC11, which mediates drug efflux, are increased in cells with AEG-1/MTDH/LYRIC overexpression (Yoo et al., 2009a). The mechanism underlying the AEG-1/MTDH/LYRIC-mediated increase in ABCC11 is likely different from that of MDR1 (i.e., polysome loading) given that MDR1 mRNA levels were unchanged in response to alterations in AEG-1/MTDH/LYRIC expression (Yoo et al., 2010).

C. Endometrial cancer

Our group has also used microarray to survey the downstream genes under the control of AEG-1/MTDH/LYRIC in endometrial cancer cells. Our data identified calbindin 1 (CALB1), a calcium binding protein involved in phosphatidylinositol metabolism, and the lectin galectin-1 among the downstream genes regulated by AEG-1/MTDH/LYRIC (Meng 2011b). Both CALB1 (Sun et al., 2011) and galectin-1 (Camby et al., 2006) have been previously been linked to pro-survival signaling pathways. Taken with the gene array data in breast cancer and HCC, these results indicate that AEG-1/MTDH/LYRIC regulates different downstream genes depending on the cellular context. Thus, a mechanistic understanding of AEG-1/MTDH/LYRIC in each distinct cancer type may be necessary in order to identify downstream pathways that can be targeted to overcome the cellular effects of AEG-1/MTDH/LYRIC.

IV. Potential contribution of genes located near AEG-1/MTDH/LYRIC on chromosome 8q22 locus to cancer drug resistance

AEG-1/MTDH/LYRIC overexpression in 40% of breast cancer patients is attributed to a genomic gain of chromosome 8q22 (Hu et al., 2009a). In addition to AEG-1/MTDH/LYRIC, other genes in this region have been associated with a resistant phenotype, including 14-3-3 ζ , lysosomal protein transmembrane 4 β (LPTM4B), E3 identified by differential display (EDD1) and grainyhead-like 3 (GRHL2) (Figure 2).

Increased expression of 14-3-3 ζ (also YWHAZ) correlates with resistance to tamoxifen and a shortened time to recurrence (Bergamaschi et al., 2013; Bergamaschi and Katzenellenbogen, 2012). As a member of the 14-3-3 family, 14-3-3 ζ regulates multiple cellular proteins by altering protein conformation, stability, activity, localization, and complex formation. Some 14-3-3 isoforms act as tumor suppressors, whereas others, including 14-3-3 ζ , act as oncogenes. In addition, some 14-3-3 isoforms have been implicated in chemoresistance (Sinha et al., 2000; Vazquez et al., 2010), with 14-3-3 ζ being one such isoform (Murata et al., 2012). Exposure to tamoxifen promotes increased expression of 14-3-3 ζ due to downregulation of miRNA-451, which specifically targets 14-3-3 ζ (Bergamaschi and Katzenellenbogen, 2012). The increase in 14-3-3 ζ results in resistance to tamoxifen (Figure 2) and, not surprisingly, levels of 14-3-3 ζ and miR-451 are inversely correlated in tamoxifen-resistant breast cancer cells (Bergamaschi and Katzenellenbogen, 2012).

In addition to early disease recurrence after tamoxifen treatment, amplification of 14-3-3 ζ as well as LPTM4B is associated with early disease recurrence after anthracycline-based chemotherapy (Li et al., 2011b). LPTM4B mediates multidrug resistance by promoting drug efflux for a variety of drugs, including doxorubicin, paclitaxel and cisplatin, in a mechanism that includes interactions with MDR1 (Li et al., 2010a). LPTM4B also interacts with the p85 α regulatory subunit of PI3K to activate PI3K/Akt signaling (Li et al., 2010a). Knockdown of either of 14-3-3 ζ or LPTM4B sensitizes cancer cells to anthracyclines, whereas overexpression produces the anticipated resistance (Li et al., 2010b). In the setting of LPTM4B overexpression, doxorubicin nuclear localization is delayed. In addition to drug efflux, LPTM4B plays a key role in lysosomes during autophagic maturation (Li et al., 2011b). Consistent with its role in cell survival through PI3K/Akt, overexpression of LPTM4B promotes protective autophagy and cell survival under stress conditions, whereas LPTM4B knockdown inhibits maturation of the autophagosome, thereby restoring sensitive to chemotherapy (Li et al., 2011b). It is of note that AEG-1/MTDH/LYRIC promotes drug resistance through many of these same mechanisms, i.e., activation of PI3K/Akt and induction of protective autophagy.

Deregulation of apoptotic pathways, such as death receptor pathways, is frequently associated with cancer recurrence. An shRNA screen identified several genes including two genes that are adjacent to AEG-1/MTDH/LYRIC, EDD1 (also called UBR5) and GRHL2, as potential targets to restore apoptosis induced by death receptor pathways (Dompe et al., 2011). While it is intriguing to postulate that evasion of apoptosis may be one mechanism for increased drug resistance in tumors with amplified chromosome 8q22, GRHL2 was recently found to suppress oncogenic transformation (Cieply et al., 2012).

V. Conclusions and future perspectives

AEG-1/MTDH/LYRIC overexpression contributes to cancer metastasis and drug resistance at multiple levels, including control of transcription, mRNA stability, and translation. A recent study has reported an association between AEG-1/MTDH/LYRIC overexpression and resistance to γ -radiation in cervical cancer (Zhao et al., 2012). It will be interesting to

determine the pathways by which AEG-1/MTDH/LYRIC mediates this effect, and whether they are the same or different from those that mediate resistance to DNA damaging chemotherapy (i.e., anthracyclines, cisplatin). This will be particularly informative for types of cancer in which radiation is the frontline therapy.

While no mutations have been reported in AEG-1/MTDH/LYRIC in cancer specimens, a very recent report identified a polymorphism in AEG-1/MTDH/LYRIC (-470G>A) that predicts for development of ovarian cancer (Yuan et al., 2012). Specifically, patients with the GG allele have an increased risk of developing cancer, though no correlation between the presence of the polymorphism and AEG-1/MTDH/LYRIC overexpression were detected. Future studies should explore whether this polymorphism might serve as a biomarker for drug resistance in addition to tumorigenic potential.

We are only beginning to understand the function of AEG-1/MTDH/LYRIC in normal biological processes. Gene knockout and transgenic mouse models, such as the recently published hepatocyte-specific AEG-1/MTDH/LYRIC transgenic mouse (Srivastava et al 2012), will provide new insights into AEG-1/MTDH/LYRIC in physiology and pathology. AEG-1/MTDH/LYRIC has no canonical functional domains besides nuclear localization sequences, protein interaction regions, and the putative RNA binding motifs identified by our group. The lack of obvious functional domains precludes development of small molecule inhibitors that target AEG-1/MTDH/LYRIC function. Specific inhibitors that disrupt the interactions between AEG-1/MTDH/LYRIC and other proteins or mRNAs have the potential to overcome AEG-1/MTDH/LYRIC-mediated drug resistance. Alternatively, a structural characterization of AEG-1/MTDH/LYRIC may facilitate the development of specific AEG-1/MTDH/LYRIC inhibitors.

It will also be informative to better understand how AEG-1/MTDH/LYRIC activity is regulated. AEG-1/MTDH/LYRIC is present in both the cytoplasm and nucleus and seems to have independent functions based on its localization. Intracellular distribution of AEG-1/MTDH/LYRIC has been shown to be regulated by ubiquitination (Thirkettle et al., 2009a). In addition, several phospho-proteomic studies have identified AEG-1/MTDH/LYRIC phosphorylation on seven sites (S84, T143, S298, S308, S415, S426, and S496) (Dephoure et al., 2008; Olsen et al., 2006). These sites are predicted substrates for MAPK, polo-like kinase 1 (PLK1) and ataxia telangiectasia mutated (ATM), the latter indicating a potential involvement of AEG-1/MTDH/LYRIC in DNA damage response. However, the effect of these post-translation modifications on AEG-1/MTDH/LYRIC-mediated drug resistance is unknown. Understanding their functional significance may provide valuable information in reaching the goal of an AEG-1/MTDH/LYRIC-targeted agent.

Acknowledgments

This work was partially supported by NIH Grant R01CA99908-7 to K.K.L., the Department of Obstetrics and Gynecology Research Development Fund, and Institutional Research Grant Number IRG-77-004-31 from the American Cancer Society to XM, administered through the Holden Comprehensive Cancer Center at the University of Iowa.

References

- Ahmad A, Sakr WA, Rahman KM. Novel targets for detection of cancer and their modulation by chemopreventive natural compounds. *Front Biosci (Elite Ed)*. 2012; 4:410–425. [PubMed: 22201883]
- Bergamaschi A, Frasar J, Borgen K, Stanculescu A, Johnson P, Rowland K, Wiley EL, Katzenellenbogen BS. 14-3-3zeta as a predictor of early time to recurrence and distant metastasis in

- hormone receptor-positive and -negative breast cancers. *Breast cancer research and treatment*. 2013; 137:689–696. [PubMed: 23271328]
- Bergamaschi A, Katzenellenbogen BS. Tamoxifen downregulation of miR-451 increases 14-3-3zeta and promotes breast cancer cell survival and endocrine resistance. *Oncogene*. 2012; 31:39–47. [PubMed: 21666713]
- Bergamaschi A, Kim YH, Wang P, Sorlie T, Hernandez-Boussard T, Lonning PE, Tibshirani R, Borresen-Dale AL, Pollack JR. Distinct patterns of DNA copy number alteration are associated with different clinicopathological features and gene-expression subtypes of breast cancer. *Genes, chromosomes & cancer*. 2006; 45:1033–1040. [PubMed: 16897746]
- Bhutia SK, Kegelmann TP, Das SK, Azab B, Su ZZ, Lee SG, Sarkar D, Fisher PB. Astrocyte elevated gene-1 induces protective autophagy. *Proceedings of the National Academy of Sciences of the United States of America*. 2010; 107:22243–22248. [PubMed: 21127263]
- Bierkens M, Krijgsman O, Wilting SM, Bosch L, Jaspers A, Meijer GA, Meijer CJ, Snijders PJ, Ylstra B, Steenbergen RD. Focal aberrations indicate EYA2 and hsa-miR-375 as oncogene and tumor suppressor in cervical carcinogenesis. *Genes, chromosomes & cancer*. 2013; 52:56–68. [PubMed: 22987659]
- Blanco MA, Aleckovic M, Hua Y, Li T, Wei Y, Xu Z, Cristea IM, Kang Y. Identification of staphylococcal nuclease domain-containing 1 (SND1) as a Metadherin-interacting protein with metastasis-promoting functions. *The Journal of biological chemistry*. 2011; 286:19982–19992. [PubMed: 21478147]
- Brown DM, Ruoslahti E. Metadherin, a cell surface protein in breast tumors that mediates lung metastasis. *Cancer cell*. 2004; 5:365–374. [PubMed: 15093543]
- Camby I, Le Mercier M, Lefranc F, Kiss R. Galectin-1: a small protein with major functions. *Glycobiology*. 2006; 16:137R–157R.
- Chang C, Shi H, Wang C, Wang J, Geng N, Jiang X, Wang X. Correlation of microRNA-375 downregulation with unfavorable clinical outcome of patients with glioma. *Neurosci Lett*. 2012; 531:204–208. [PubMed: 23103713]
- Chang TC, Yu D, Lee YS, Wentzel EA, Arking DE, West KM, Dang CV, Thomas-Tikhonenko A, Mendell JT. Widespread microRNA repression by Myc contributes to tumorigenesis. *Nature genetics*. 2008; 40:43–50. [PubMed: 18066065]
- Chen M, Zhang J, Manley JL. Turning on a fuel switch of cancer: hnRNP proteins regulate alternative splicing of pyruvate kinase mRNA. *Cancer research*. 2010; 70:8977–8980. [PubMed: 20978194]
- Chen ZS, Tiwari AK. Multidrug resistance proteins (MRPs/ABCCs) in cancer chemotherapy and genetic diseases. *Febs J*. 2011; 278:3226–3245. [PubMed: 21740521]
- Cieply B, Riley Pt, Pifer PM, Widmeyer J, Addison JB, Ivanov AV, Denvir J, Frisch SM. Suppression of the epithelial-mesenchymal transition by Grainyhead-like-2. *Cancer research*. 2012; 72:2440–2453. [PubMed: 22379025]
- Dai X, Chiang Y, Wang Z, Song Y, Lu C, Gao P, Xu H. Expression levels of microRNA-375 in colorectal carcinoma. *Mol Med Report*. 2012; 5:1299–1304.
- Dephoure N, Zhou C, Villen J, Beausoleil SA, Bakalarski CE, Elledge SJ, Gygi SP. A quantitative atlas of mitotic phosphorylation. *Proceedings of the National Academy of Sciences of the United States of America*. 2008; 105:10762–10767. [PubMed: 18669648]
- Dompe N, Rivers CS, Li L, Cordes S, Schwickart M, Punnoose EA, Amler L, Seshagiri S, Tang J, Modrusan Z, Davis DP. A whole-genome RNAi screen identifies an 8q22 gene cluster that inhibits death receptor-mediated apoptosis. *Proceedings of the National Academy of Sciences of the United States of America*. 2011; 108:E943–951. [PubMed: 21949371]
- Emdad L, Lee SG, Su ZZ, Jeon HY, Boukerche H, Sarkar D, Fisher PB. Astrocyte elevated gene-1 (AEG-1) functions as an oncogene and regulates angiogenesis. *Proceedings of the National Academy of Sciences of the United States of America*. 2009; 106:21300–21305. [PubMed: 19940250]
- Emdad L, Sarkar D, Su ZZ, Randolph A, Boukerche H, Valerie K, Fisher PB. Activation of the nuclear factor kappaB pathway by astrocyte elevated gene-1: implications for tumor progression and metastasis. *Cancer research*. 2006; 66:1509–1516. [PubMed: 16452207]

- Finnegan EF, Pasquinelli AE. MicroRNA biogenesis: regulating the regulators. *Critical reviews in biochemistry and molecular biology*. 2013; 48:51–68. [PubMed: 23163351]
- Galardi S, Mercatelli N, Farace MG, Ciafre SA. NF- κ B and c-Jun induce the expression of the oncogenic miR-221 and miR-222 in prostate carcinoma and glioblastoma cells. *Nucleic Acids Res*. 2011; 39:3892–3902. [PubMed: 21245048]
- Grant TJ, Bishop JA, Christadore LM, Barot G, Chin HG, Woodson S, Kavouris J, Siddiq A, Gredler R, Shen XN, et al. Antiproliferative small-molecule inhibitors of transcription factor LSF reveal oncogene addiction to LSF in hepatocellular carcinoma. *Proceedings of the National Academy of Sciences of the United States of America*. 2012; 109:4503–4508. [PubMed: 22396589]
- He XX, Chang Y, Meng FY, Wang MY, Xie QH, Tang F, Li PY, Song YH, Lin JS. MicroRNA-375 targets AEG-1 in hepatocellular carcinoma and suppresses liver cancer cell growth in vitro and in vivo. *Oncogene*. 2012; 31:3357–3369. [PubMed: 22056881]
- Holleman A, Chung I, Olsen RR, Kwak B, Mizokami A, Saijo N, Parissenti A, Duan Z, Voest EE, Zetter BR. miR-135a contributes to paclitaxel resistance in tumor cells both in vitro and in vivo. *Oncogene*. 2011; 30:4386–4398. [PubMed: 21552288]
- Holoch PA, Griffith TS. TNF-related apoptosis-inducing ligand (TRAIL): a new path to anti-cancer therapies. *European journal of pharmacology*. 2009; 625:63–72. [PubMed: 19836385]
- Hu G, Chong RA, Yang Q, Wei Y, Blanco MA, Li F, Reiss M, Au JL, Haffty BG, Kang Y. MTDH activation by 8q22 genomic gain promotes chemoresistance and metastasis of poor-prognosis breast cancer. *Cancer cell*. 2009a; 15:9–20. [PubMed: 19111877]
- Hu G, Wei Y, Kang Y. The multifaceted role of MTDH/AEG-1 in cancer progression. *Clinical cancer research : an official journal of the American Association for Cancer Research*. 2009b; 15:5615–5620. [PubMed: 19723648]
- Isozaki Y, Hoshino I, Nohata N, Kinoshita T, Akutsu Y, Hanari N, Mori M, Yoneyama Y, Akanuma N, Takeshita N, et al. Identification of novel molecular targets regulated by tumor suppressive miR-375 induced by histone acetylation in esophageal squamous cell carcinoma. *Int J Oncol*. 2012; 41:985–994. [PubMed: 22752059]
- Kang DC, Su ZZ, Sarkar D, Emdad L, Volsky DJ, Fisher PB. Cloning and characterization of HIV-1-inducible astrocyte elevated gene-1, AEG-1. *Gene*. 2005; 353:8–15. [PubMed: 15927426]
- Kikuno N, Shiina H, Urakami S, Kawamoto K, Hirata H, Tanaka Y, Place RF, Pookot D, Majid S, Igawa M, Dahiya R. Knockdown of astrocyte-elevated gene-1 inhibits prostate cancer progression through upregulation of FOXO3a activity. *Oncogene*. 2007; 26:7647–7655. [PubMed: 17563745]
- Kinoshita T, Hanazawa T, Nohata N, Okamoto Y, Seki N. The functional significance of microRNA-375 in human squamous cell carcinoma: aberrant expression and effects on cancer pathways. *J Hum Genet*. 2012; 57:556–563. [PubMed: 22718022]
- Kong X, Moran MS, Zhao Y, Yang Q. Inhibition of metadherin sensitizes breast cancer cells to AZD6244. *Cancer biology & therapy*. 2012; 13:43–49. [PubMed: 22336587]
- Lee SG, Jeon HY, Su ZZ, Richards JE, Vozhilla N, Sarkar D, Van Maerken T, Fisher PB. Astrocyte elevated gene-1 contributes to the pathogenesis of neuroblastoma. *Oncogene*. 2009; 28:2476–2484. [PubMed: 19448665]
- Lee SG, Su ZZ, Emdad L, Sarkar D, Franke TF, Fisher PB. Astrocyte elevated gene-1 activates cell survival pathways through PI3K-Akt signaling. *Oncogene*. 2008; 27:1114–1121. [PubMed: 17704808]
- Levenson JD, Koskinen PJ, Orrico FC, Rainio EM, Jalkanen KJ, Dash AB, Eisenman RN, Ness SA. Pim-1 kinase and p100 cooperate to enhance c-Myb activity. *Molecular cell*. 1998; 2:417–425. [PubMed: 9809063]
- Li C, Li R, Song H, Wang D, Feng T, Yu X, Zhao Y, Liu J, Wang Y, Geng J. Significance of AEG-1 expression in correlation with VEGF, microvessel density and clinicopathological characteristics in triple-negative breast cancer. *J Surg Oncol*. 2011a; 103:184–192. [PubMed: 21259255]
- Li C, Li Y, Wang X, Wang Z, Cai J, Wang L, Zhao Y, Song H, Meng X, Ning X, et al. Elevated expression of astrocyte elevated gene-1 (AEG-1) is correlated with cisplatin-based chemoresistance and shortened outcome in patients with stages III-IV serous ovarian carcinoma. *Histopathology*. 2012a; 60:953–963. [PubMed: 22372608]

- Li L, Wei XH, Pan YP, Li HC, Yang H, He QH, Pang Y, Shan Y, Xiong FX, Shao GZ, Zhou RL. LAPTM4B: a novel cancer-associated gene motivates multidrug resistance through efflux and activating PI3K/AKT signaling. *Oncogene*. 2010a; 29:5785–5795. [PubMed: 20711237]
- Li Y, Jiang Q, Xia N, Yang H, Hu C. Decreased Expression of MicroRNA-375 in Nonsmall Cell Lung Cancer and its Clinical Significance. *J Int Med Res*. 2012b; 40:1662–1669. [PubMed: 23206448]
- Li Y, Zhang Q, Tian R, Wang Q, Zhao JJ, Iglehart JD, Wang ZC, Richardson AL. Lysosomal transmembrane protein LAPTM4B promotes autophagy and tolerance to metabolic stress in cancer cells. *Cancer research*. 2011b; 71:7481–7489. [PubMed: 22037872]
- Li Y, Zou L, Li Q, Haibe-Kains B, Tian R, Desmedt C, Sotiropoulos C, Szallasi Z, Iglehart JD, Richardson AL, Wang ZC. Amplification of LAPTM4B and YWHAZ contributes to chemotherapy resistance and recurrence of breast cancer. *Nat Med*. 2010b; 16:214–218. [PubMed: 20098429]
- Liu S, Guo W, Shi J, Li N, Yu X, Xue J, Fu X, Chu K, Lu C, Zhao J, et al. MicroRNA-135a contributes to the development of portal vein tumor thrombus by promoting metastasis in hepatocellular carcinoma. *J Hepatol*. 2012; 56:389–396. [PubMed: 21888875]
- Lu L, Schwartz P, Scarampi L, Rutherford T, Canuto EM, Yu H, Katsaros D. MicroRNA let-7a: a potential marker for selection of paclitaxel in ovarian cancer management. *Gynecologic oncology*. 2011; 122:366–371. [PubMed: 21571355]
- Lum JJ, Bauer DE, Kong M, Harris MH, Li C, Lindsten T, Thompson CB. Growth factor regulation of autophagy and cell survival in the absence of apoptosis. *Cell*. 2005; 120:237–248. [PubMed: 15680329]
- Meng F, Luo C, Ma L, Hu Y, Lou G. Clinical significance of astrocyte elevated gene-1 expression in human epithelial ovarian carcinoma. *Int J Gynecol Pathol*. 2011a; 30:145–150. [PubMed: 21293286]
- Meng X, Brachova P, Yang S, Xiong Z, Zhang Y, Thiel KW, Leslie KK. Knockdown of MTDH sensitizes endometrial cancer cells to cell death induction by death receptor ligand TRAIL and HDAC inhibitor LBH589 co-treatment. *PloS one*. 2011b; 6:e20920. [PubMed: 21687633]
- Meng X, Zhu D, Yang S, Wang X, Xiong Z, Zhang Y, Brachova P, Leslie KK. Cytoplasmic Metadherin (MTDH) provides survival advantage under conditions of stress by acting as RNA-binding protein. *The Journal of biological chemistry*. 2012; 287:4485–4491. [PubMed: 22199357]
- Murata T, Takayama K, Urano T, Fujimura T, Ashikari D, Obinata D, Horie-Inoue K, Takahashi S, Ouchi Y, Homma Y, Inoue S. 14-3-3zeta, a novel androgen-responsive gene, is upregulated in prostate cancer and promotes prostate cancer cell proliferation and survival. *Clin Cancer Res*. 2012; 18:5617–5627. [PubMed: 22904106]
- Nestal de Moraes G, Souza PS, Costas FC, Vasconcelos FC, Reis FR, Maia RC. The Interface between BCR-ABL-Dependent and -Independent Resistance Signaling Pathways in Chronic Myeloid Leukemia. *Leuk Res Treatment*. 2012; 2012:671702. [PubMed: 23259070]
- Nohata N, Hanazawa T, Kikkawa N, Mutallip M, Sakurai D, Fujimura L, Kawakami K, Chiyomaru T, Yoshino H, Enokida H, et al. Tumor suppressive microRNA-375 regulates oncogene AEG-1/MTDH in head and neck squamous cell carcinoma (HNSCC). *J Hum Genet*. 2011; 56:595–601. [PubMed: 21753766]
- Olsen JV, Blagoev B, Gnäd F, Macek B, Kumar C, Mortensen P, Mann M. Global, in vivo, and site-specific phosphorylation dynamics in signaling networks. *Cell*. 2006; 127:635–648. [PubMed: 17081983]
- Poon TC, Wong N, Lai PB, Rattray M, Johnson PJ, Sung JJ. A tumor progression model for hepatocellular carcinoma: bioinformatic analysis of genomic data. *Gastroenterology*. 2006; 131:1262–1270. [PubMed: 17030195]
- Qian BJ, Yan F, Li N, Liu QL, Lin YH, Liu CM, Luo YP, Guo F, Li HZ. MTDH/AEG-1-based DNA vaccine suppresses lung metastasis and enhances chemosensitivity to doxorubicin in breast cancer. *Cancer Immunol Immunother*. 2011; 60:883–893. [PubMed: 21400023]
- Rodriguez Lajusticia L, Martin Jimenez M, Lopez-Tarruella Cobo S. Endocrine therapy of metastatic breast cancer. *Clin Transl Oncol*. 2008; 10:462–467. [PubMed: 18667376]
- Santhekadur PK, Das SK, Gredler R, Chen D, Srivastava J, Robertson C, Baldwin AS Jr, Fisher PB, Sarkar D. Multifunction protein staphylococcal nuclease domain containing 1 (SND1) promotes tumor angiogenesis in human hepatocellular carcinoma through novel pathway that involves

- nuclear factor kappaB and miR-221. *The Journal of biological chemistry*. 2012; 287:13952–13958. [PubMed: 22396537]
- Sarkar D, Park ES, Emdad L, Lee SG, Su ZZ, Fisher PB. Molecular basis of nuclear factor-kappaB activation by astrocyte elevated gene-1. *Cancer research*. 2008; 68:1478–1484. [PubMed: 18316612]
- Sinha P, Kohl S, Fischer J, Hutter G, Kern M, Kottgen E, Dietel M, Lage H, Schnolzer M, Schadendorf D. Identification of novel proteins associated with the development of chemoresistance in malignant melanoma using two-dimensional electrophoresis. *Electrophoresis*. 2000; 21:3048–3057. [PubMed: 11001322]
- Srivastava J, Siddiq A, Emdad L, Santhekadur PK, Chen D, Gredler R, Shen XN, Dumur CL, Hylemon PB, Mukhopadhyay ND, et al. Astrocyte elevated gene-1 (AEG-1) promotes hepatocarcinogenesis: Novel insights from a mouse model. *Hepatology*. 2012a
- 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*. 2012b; 56:1782–1791. [PubMed: 22689379]
- Sun S, Li F, Gao X, Zhu Y, Chen J, Zhu X, Yuan H, Gao D. Calbindin-D28K inhibits apoptosis in dopaminergic neurons by activation of the PI3-kinase-Akt signaling pathway. *Neuroscience*. 2011; 199:359–367. [PubMed: 22020319]
- Tamada M, Suematsu M, Saya H. Pyruvate kinase m2: multiple faces for conferring benefits on cancer cells. *Clinical cancer research : an official journal of the American Association for Cancer Research*. 2012; 18:5554–5561. [PubMed: 23071357]
- Thirkettle HJ, Girling J, Warren AY, Mills IG, Sahadevan K, Leung H, Hamdy F, Whitaker HC, Neal DE. LYRIC/AEG-1 is targeted to different subcellular compartments by ubiquitinylation and intrinsic nuclear localization signals. *Clinical cancer research : an official journal of the American Association for Cancer Research*. 2009a; 15:3003–3013. [PubMed: 19383828]
- Thirkettle HJ, Mills IG, Whitaker HC, Neal DE. Nuclear LYRIC/AEG-1 interacts with PLZF and relieves PLZF-mediated repression. *Oncogene*. 2009b; 28:3663–3670. [PubMed: 19648967]
- Tokunaga E, Nakashima Y, Yamashita N, Hisamatsu Y, Okada S, Akiyoshi S, Aishima S, Kitao H, Morita M, Maehara Y. Overexpression of metadherin/MTDH is associated with an aggressive phenotype and a poor prognosis in invasive breast cancer. *Breast Cancer*. 2012
- Totary-Jain H, Sanoudou D, Ben-Dov IZ, Dautriche CN, Guarnieri P, Marx SO, Tuschl T, Marks AR. Reprogramming of the microRNA transcriptome mediates resistance to rapamycin. *The Journal of biological chemistry*. 2013
- Unsworth H, Raguz S, Edwards HJ, Higgins CF, Yague E. mRNA escape from stress granule sequestration is dictated by localization to the endoplasmic reticulum. *Faseb J*. 2010; 24:3370–3380. [PubMed: 20453113]
- Valineva T, Yang J, Palovuori R, Silvennoinen O. The transcriptional co-activator protein p100 recruits histone acetyltransferase activity to STAT6 and mediates interaction between the CREB-binding protein and STAT6. *The Journal of biological chemistry*. 2005; 280:14989–14996. [PubMed: 15695802]
- Vazquez A, Grochola LF, Bond EE, Levine AJ, Taubert H, Muller TH, Wurl P, Bond GL. Chemosensitivity profiles identify polymorphisms in the p53 network genes 14-3-3tau and CD44 that affect sarcoma incidence and survival. *Cancer research*. 2010; 70:172–180. [PubMed: 19996285]
- Walczak H, Miller RE, Ariail K, Gliniak B, Griffith TS, Kubin M, Chin W, Jones J, Woodward A, Le T, et al. Tumoricidal activity of tumor necrosis factor-related apoptosis-inducing ligand in vivo. *Nat Med*. 1999; 5:157–163. [PubMed: 9930862]
- Wang C, Yang Q. Astrocyte elevated gene-1 and breast cancer (Review). *Oncol Lett*. 2011; 2:399–405. [PubMed: 22866094]
- Ward A, Balwierz A, Zhang JD, Kublbeck M, Pawitan Y, Hielscher T, Wiemann S, Sahin O. Re-expression of microRNA-375 reverses both tamoxifen resistance and accompanying EMT-like properties in breast cancer. *Oncogene*. 2012
- Weissbach R, Scadden AD. Tudor-SN and ADAR1 are components of cytoplasmic stress granules. *Rna*. 2012; 18:462–471. [PubMed: 22240577]

- Wilson MS, Brosens JJ, Schwenen HD, Lam EW. FOXO and FOXM1 in cancer: the FOXO-FOXM1 axis shapes the outcome of cancer chemotherapy. *Curr Drug Targets*. 2011; 12:1256–1266. [PubMed: 21443467]
- Ying Z, Li J, Li M. Astrocyte elevated gene 1: biological functions and molecular mechanism in cancer and beyond. *Cell Biosci*. 2011; 1:36. [PubMed: 22060137]
- Yoo BK, Chen D, Su ZZ, Gredler R, Yoo J, Shah K, Fisher PB, Sarkar D. Molecular mechanism of chemoresistance by astrocyte elevated gene-1. *Cancer research*. 2010; 70:3249–3258. [PubMed: 20388796]
- Yoo BK, Emdad L, Lee SG, Su ZZ, Santhekadur P, Chen D, Gredler R, Fisher PB, Sarkar D. Astrocyte Elevated Gene-1 (AEG-1): A multifunctional regulator of normal and abnormal physiology. *Pharmacology & therapeutics*. 2011a
- 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. *The Journal of clinical investigation*. 2009a; 119:465–477. [PubMed: 19221438]
- Yoo BK, Gredler R, Chen D, Santhekadur PK, Fisher PB, Sarkar D. c-Met activation through a novel pathway involving osteopontin mediates oncogenesis by the transcription factor LSF. *J Hepatol*. 2011b; 55:1317–1324. [PubMed: 21703197]
- Yoo BK, Gredler R, Vozhilla N, Su ZZ, Chen D, Forcier T, Shah K, Saxena U, Hansen U, Fisher PB, Sarkar D. Identification of genes conferring resistance to 5-fluorouracil. *Proceedings of the National Academy of Sciences of the United States of America*. 2009b; 106:12938–12943. [PubMed: 19622726]
- Yoo BK, Santhekadur PK, Gredler R, Chen D, Emdad L, Bhutia S, Pannell L, Fisher PB, Sarkar D. Increased RNA-induced silencing complex (RISC) activity contributes to hepatocellular carcinoma. *Hepatology*. 2011c; 53:1538–1548. [PubMed: 21520169]
- Yuan C, Li X, Yan S, Yang Q, Liu X, Kong B. The MTDH (–470G>A) Polymorphism Is Associated with Ovarian Cancer Susceptibility. *PloS one*. 2012; 7:e51561. [PubMed: 23240043]
- Zhao Y, Moran MS, Yang Q, Liu Q, Yuan C, Hong S, Kong B. Metadherin regulates radioresistance in cervical cancer cells. *Oncol Rep*. 2012; 27:1520–1526. [PubMed: 22367022]

Figure 1. Molecular mechanisms of chemoresistance mediated by metadherin (AEG-1/MTDH/LYRIC) in cancer

In the cytoplasm, AEG-1/MTDH/LYRIC has been proposed to regulate mRNA stability, loading in the polysome, and protein translation, putatively by interacting with distinct mRNA sequences (Yoo et al., 2010; Meng et al., 2012). Cytoplasmic AEG-1/MTDH/LYRIC also serves as a component of RISC by interacting with SND1 (Blanco et al., 2011; Yoo, Santhekadur, et al., 2011). AEG-1/MTDH/LYRIC augments the PI3K/Akt signaling pathway to inhibit FOXO1 and FOXO3a-mediated apoptosis (Kikuno et al., 2007; Lee et al., 2008). Protective autophagy is induced via increased activation of AMPK and ATG-5 (Bhutia et al., 2010). In the nucleus, AEG-1/MTDH/LYRIC interacts with PLZF and releases suppression of target genes, including c-myc (Thirkettle, Mills, et al., 2009). In a feedback loop, c-myc increases transcription of AEG-1/MTDH/LYRIC and suppress expression of microRNAs (Wilson et al., 2011). Increased AEG-1/MTDH/LYRIC expression stabilizes the p50/p65 complex of NFκB (Emdad et al., 2006), inducing complex translocation to the nucleus. Downstream genes, including ALDH3A1, MET, LSF, DPYD and MDR1, are up-regulated by AEG-1/MTDH/LYRIC and thus mediate chemoresistance. Dotted lines indicate pathways yet to be fully characterized.

Figure 2. Mechanisms of drug resistance induced by chromosome 8q22 amplification

Chromosome 8q22 amplification results in increased expression of AEG-1/MTDH/LYRIC, 14-3-3 ζ , LAPTM4B, EDD1 and GRHL2, and each of these genes have been implicated in drug resistance. Tamoxifen resistance correlates with increased expression of AEG-1/MTDH/LYRIC and 14-3-3 ζ and decreased expression of miR-375 and miR-451.

Table 1

Various cancers in which AEG-1/MTDH/LYRIC overexpression has been associated with drug resistance.

Cancer sites	Drug	References
Breast Cancer	doxorubicin, paclitaxel, cisplatin tamoxifen AZD6244	Hu et al., 2009a Ward et al., 2012 Kong et al., 2012
HCC	5-fluorouracil doxorubicin	Yoo et al., 2009 Yoo et al., 2010
Ovarian Cancer	cisplatin	Li et al., 2012a
Endometrial Cancer	TRAIL, HDAC inhibitor, Mitomycin C, BIBF1120	Meng et al., 2011b Meng et al., 2012
Neuroblastoma	cisplatin, doxorubicin	Liu et al., 2009

Table 2

Representative mRNAs that associate with AEG-1/MTDH/LYRIC. The full list of RNAs associated with AEG-1/MTDH/LYRIC is provided in Supplementary Tables 2–5 of (Meng et al., 2012).

Gene	Function
<i>WHSC1L1</i>	histone methyltransferase
<i>KDM6A</i>	histone demethylase
<i>PDCD11</i>	programmed cell death
<i>FANCI</i>	DNA repair
<i>FANCA</i>	DNA repair
<i>FANCD2</i>	DNA repair
<i>FANCC</i>	DNA repair
<i>OSMR</i>	oncostatin M receptor
<i>POLQ</i>	DNA polymerase
<i>MMP16</i>	matrix metalloproteinase
<i>DDX6</i>	RNA helicase
<i>DDX21</i>	RNA helicase
<i>DDX60</i>	RNA helicase
<i>WWP1</i>	E3 ubiquitin ligase
<i>PPP2R1B</i>	Protein phosphatase 2 regulatory subunit A beta
<i>ZNF217</i>	Transcription factor

* Abbreviations: *WHSC1L1*, Wolf-Hirschhorn syndrome candidate 1-like 1; *KDM6A*, lysine (K)-specific demethylase 6A; FANC, Fanconi anemia, complementation group; DDX, DEAD (Asp-Glu-Ala-Asp) box helicase; WWP1, WW domain containing E3 ubiquitin protein ligase 1; ZNF217, zinc finger protein 217.