Molecular Pathways

The Multifaceted Role of MTDH/AEG-1 in Cancer Progression

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Abstract Cancer is the result of the progressive acquisition of multiple malignant traits through the accumulation of genetic or epigenetic alterations. Recent studies have established a functional role of MTDH (Metadherin)/AEG-1 (Astrocyte Elevated Gene 1) in several crucial aspects of tumor progression, including transformation, evasion of apoptosis, invasion, metastasis, and chemoresistance. Overexpression of MTDH/AEG-1 is frequently observed in melanoma, glioma, neuroblastoma, and carcinomas of breast, prostate, liver, and esophagus and is correlated with poor clinical outcomes. MTDH/AEG-1 functions as a downstream mediator of the transforming activity of oncogenic Ha-Ras and c-Myc. Furthermore, MTDH/AEG-1 overexpression activates the PI3K/Akt, nuclear factor κB (NFκB), and Wnt/β-catenin signaling pathways to stimulate proliferation, invasion, cell survival, and chemoresistance. The lung-homing domain of MTDH/AEG-1 also mediates the adhesion of tumor cells to the vasculature of distant organs and promotes metastasis. These findings suggest that therapeutic targeting of MTDH/AEG-1 may simultaneously suppress tumor growth, block metastasis, and enhance the efficacy of chemotherapeutic treatments. (Clin Cancer Res 2009;15(18):5615–20)

Background Cancer progression is driven by the accumulation of numerous genetic and epigenetic alterations that promote tumor initiation, expansion, and metastasis (1–3). In the past few decades, massive efforts in cancer research have led to the identification of a seemingly exhaustive list of oncogenes, tumor suppressors, and signal pathways that are potential targets for anticancer therapeutics. Metadherin (MTDH), also known as AEG-1, and Lyric, a novel gene that was cloned only 5 years ago, has emerged in recent years as a potentially crucial mediator of tumor malignancy and a key converging point of a complex network of oncogenic signaling pathways (4, 5).

Cloning and molecular characteristics. MTDH/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 (6). Full-length MTDH/AEG-1 cDNA was subsequently cloned by four independent groups (7–10). Brown and colleagues used a phage display screen to identify a lung-homing peptide in MTDH that allowed the identification of lysine-rich CEACAM-1 co-isolated protein (Lyric) that colocalizes with the tight junction protein ZO-1 in polarized rat prostate epithelial cells (9), and as a novel transmembrane protein that is present in epithelium, endoplasmic reticulum, perinuclear regions, and nucleolus (10).

MTDH/AEG-1 orthologs were found in most vertebrate species but not in non-vertebrates. Although evolutionarily highly conserved, MTDH/AEG-1 does not have any recognizable protein domains except three putative lysine-rich nuclear localization signals (NLS). Human MTDH/AEG-1 encodes a 582-amino acid protein with a calculated molecular mass of 64 kDa. MTDH/AEG-1 is expressed in variable levels in most tissues. Antibodies against MTDH/AEG-1 often detect multiple proteins with molecular weights ranging from 75 to 80 kDa to 20 kDa, possibly because of alternative splicing and/or posttranslational modification (7–10). MTDH/AEG1 is rich in both lysine (12.3%) and serine (11.6%) residues that are targets for posttranslational modifications such as acetylation and ubiquitination of lysines (11) and phosphorylation of serine and threonine. How posttranscriptional and posttranslational modifications of MTDH/AEG-1 influence its function and localization is currently unknown.

Immunofluorescence and immunohistochemical analysis of MTDH/AEG-1 often showed perinuclear and cytoplasmic staining as well as some nuclear rim, nucleolar, and general nuclear diffuse staining in various cell types (4, 7, 9, 10). Cytoplasmic membrane localization of MTDH/AEG-1 has also been detected by immunostaining of nonpermeabilized mouse 4T1 mammary tumor cells and by FACS (8). TNF-α treatment, which up-regulates MTDH/AEG-1 expression, as well as ectopic overexpression of MTDH/AEG-1, has been shown to enhance nuclear localization of MTDH/AEG-1 in HeLa cells (12).
Nuclear localization of MTDH/AEG-1 is probably mediated by three putative lysine-rich NLS sequences, although the exact mechanism and functional significance of MTDH/AEG-1 nuclear and nucleolar translocation is still under investigation (11, 12). Several independent protein motif analysis methods predict a single transmembrane domain (amino acids 52–74) in MTDH/AEG-1. However, there is still a debate about whether MTDH/AEG-1 is a type Ib membrane protein (C-terminal in the cytoplasmic side with no signal peptide), or a type II protein (C-terminal outside) based on computational modeling (7, 9) and experiment evidence (8, 9). Although a considerable amount of work is still required to fully characterize the molecular and biochemical properties of MTDH/AEG-1, functional and clinical evidence accumulated in recent years strongly support an important role for MTDH/AEG-1 in cancer development.

Integration of oncogenic pathways. MTDH/AEG-1 contributes to several hallmarks of metastatic cancers, including aberrant proliferation, survival under stressful conditions such as serum deprivation and chemotherapy, and increased migration, invasiveness, and metastasis. Overexpression of MTDH/AEG-1 synergizes with oncogenic Ha-Ras to enhance soft-agar colony formation of immortalized melanocyte and astrocyte (7). Conversely, MTDH/AEG-1 was activated at the transcription level upon transient or stable transfection of oncogenic Ras in human fetal astrocytes (13) and MTDH/AEG-1 knockdown suppressed Ras-induced colony formation (13). Ras plays an essential role in regulating cell growth, survival, stress response, cytoskeleton reorganization, and migration by activating a number of downstream signaling pathways, including the Raf/MAPK pathway (cell proliferation), the PI3K-Akt pathway (cell survival), the Rac-Rho pathway (cytoskeletal organization), and the NFκB pathway (inflammation and survival).

MTDH/AEG-1 promotes tumor progression through the integration of multiple signaling pathways. Oncogenic Ha-Ras increases MTDH/AEG-1 expression through the activation of the PI3K/Akt pathway, which phosphorylates and inactivates GSK3β, and subsequently enhances the stabilization and binding of c-Myc to the MTDH/AEG-1 promoter. MTDH/AEG-1 can activate AKT, NFκB, and Wnt/β-catenin pathways to promote proliferation, survival, and invasion. Activation of NFκB signaling is in part mediated by the direct interaction of MTDH/AEG-1 with p65 and CBP, a general transcriptional co-activator. MTDH/AEG-1 activates the Wnt/β-catenin pathway through increasing the activity of MAPK kinases ERK and p38, which phosphorylates GSK3β and stabilized β-catenin. Furthermore, MTDH/AEG-1 increases the expression of LEF-1, a transcriptional co-factor for β-catenin. The prometastasis function of MTDH/AEG-1 is mediated by the interaction of the LHD of MTDH/AEG-1 with an unknown receptor in endothelial cells. The broad spectrum chemoresistance function of MTDH/AEG-1 is mediated by a number of downstream genes that promote the resistance to multiple chemotherapeutic agents. Proteins with direct interactions with MTDH/AEG1 are shown in green. Dotted line indicates pathways yet to be fully validated or characterized.
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Reorganization), and the Rac-JNK/p38 pathways (stress response; ref. 14–18). When inhibitors for various Ras down-stream signaling pathways were tested, only PI3K/Akt inhibitors LY294002 and PTEN were able to block the MTDH/AEG-1 promoter activation by Ras, suggesting the involvement of PI3K/Akt pathway in MTDH/AEG-1 regulation (13). Promoter mapping subsequently identified two E-boxes (binding sites for c-Myc) in the -356 to -302 region of the MTDH/AEG-1 promoter that is essential for activation by Ras (13). Linking the Akt activation to c-Myc regulation of MTDH/AEG-1 is the phosphorylation and inactivation of GSK3β, a serine-threonine kinase that phosphorylates and destabilizes c-Myc (13, 19, 20). Collectively, these data link Ras activation of MTDH/AEG-1 through PI3K-Akt-GSK3β-Myc signaling (Fig. 1) in transformed astrocytes.

Depending on the cell types tested, overexpression of MTDH/AEG-1 can activate several downstream pathways, including the Akt pathway, the nuclear factor κB (NFκB) pathway, and the Wnt/β-catenin pathway, to enhance different aspects of tumor malignancy. MTDH/AEG-1 overexpression inhibits serum starvation-induced apoptosis in normal astrocytes and fibroblasts, but not in Ras-transformed cells (21). When a panel of pathway-specific inhibitors was used to probe the downstream mediator for the prosurvival function of MTDH/AEG-1, only the PI3K inhibitor LY294002, PTEN, and dominant negative Akt were able to attenuate MTDH/AEG-1-dependent survival under serum-deprived conditions (21). MTDH/AEG-1 overexpression increases phosphorylation of Akt and GSK3β, with subsequent c-Myc stabilization and MDM2 phosphorylation, decrease of p53 and CDK inhibitor p21Cip, as well as phosphorylation of Bad, a proapoptotic member of the Bcl-2 family in astrocytes (21). These results indicate that MTDH/AEG-1-dependent cell growth and survival is mediated by Akt signaling downstream of PI3K (21). Thus, MTDH/AEG-1 is both a downstream target of Akt and an upstream activator of the PI3K-Akt pathway, although the mechanism of PI3K pathway activation by MTDH/AEG-1 remains unknown (Fig. 1).

Through the activation of Akt, MTDH/AEG-1 may affect a number of additional Akt downstream factors that are crucial for cellular proliferation and survival. MTDH/AEG-1 knockdown induces apoptosis of prostate cancer cells through the reduction of Akt activity and upregulation of FOXO3a activity (22). FOXO3a is a pro-apoptosis forkhead transcription factor that is exported from the nucleus following phosphorylation by Akt (19, 23). The activator protein 1 (AP-1) and NFκB, two other transcription regulators downstream of the PI3K/Akt pathway, are also regulated by MTDH/AEG-1 expression (12, 22). MTDH/AEG-1 enhances nuclear accumulation, DNA binding, and transcriptional activities of NFκB in Hela cells (12). The NFκB heterodimer p50 and p65 function as transcriptional factors to regulate a variety of cellular phenotypes including apoptosis, inflammation, immune response, and oncogenic proliferation (24–26). NFκB can be activated by MTDH/AEG-1 through PI3K/Akt, which activates the IKK kinase to phosphorylate and destabilize the NFκB inhibitor IκB. Alternatively, MTDH/AEG-1 has been found to physically interact with the NFκB subunit p65 directly and promote its translocation to the nucleus (12). Furthermore, MTDH/AEG-1 may bridge the interaction between p65 and CBP, a ubiquitous transcriptional co-activator of NFκB in glioma cells (Fig. 1; ref. 12, 27). In Hela cells, ectopic overexpression of MTDH/AEG-1 resulted in up-regulation of several NFκB-responsive cell adhesion molecules, such as ICAM-2 and ICAM-3, selectin E, selectin L, and selectin P ligands, as well as many other important mediators of tumor malignancy, such as IL-6, IL-8, toll-like receptors TLR-4 and TLR-5, MMP9, and transcription factors c-Jun and c-Fos (12, 22).

More recently, MTDH/AEG-1 has also been connected with the Wnt/β-catenin pathway in hepatocellular carcinoma cells through the activation of the Raf/MEK/MAPK branch of the Ras signaling pathway (28). The MTDH/AEG-1-expressing clones of human hepatocellular carcinoma HepG3 cells displayed stronger activities of several MAP kinases, including ERK and p38. These kinases phosphorylate GSK3β and increase the stability and nuclear translocation of β-catenin. Furthermore, MTDH/AEG-1 overexpression also increases the level of LEF-1, a transcription factor that interacts with β-catenin to activate gene expression in the nucleus. Specific inhibitors of the MAPK pathway are able to abolish the oncogenic effect of MTDH/AEG-1 in matrigel invasion and anchorage-independent growth (28).

Metastasis. A lung-homing domain (LHD, amino acids 378-440 in mouse or 381-443 in human) in MTDH/AEG1 was identified by Brown and colleagues in a phage display experiment to be a mediator of 4T1 mouse mammary tumor cell adhesion to lung vasculature (8). Neutralizing antibodies against LHD- or siRNA-silencing of MTDH/AEG-1 efficiently reduced lung metastasis of 4T1 cancer cells. Conversely, overexpression of MTDH/AEG-1 in the human embryonic kidney cells HEK293 led to enhanced localization of these cells to lung vasculatures (8). The endothelial adhesion and metastasis-promoting function of MTDH/AEG-1 has been validated using the MDA-MB-231 xenograft model of breast cancer metastasis (29). In this model system, MTDH/AEG-1 was found to not only promote lung metastasis, but also modestly increase bone metastasis. MTDH/AEG-1 may promote metastasis through the interaction of the LHD with an unknown receptor expressed in the surface of endothelial cells, or indirectly through the activation of signaling pathways, such as NFκB, that activate the expression of adhesion molecules.

Chemoresistance. In addition to promoting cell survival in the serum starvation condition through activating the PI3K-Akt signaling pathway (21, 22), a more general role for MTDH/AEG-1 to confer broad-spectrum chemoresistance has also been discovered recently (29, 30). Pharmacogenomic analysis of the NCI-60 panel of cancer cell lines revealed a significant correlation of MTDH/AEG-1 overexpression with the resistance of cancer cells to a broader spectrum of chemical compounds. In vitro and in vivo chemoresistance analyses showed that MTDH/AEG-1 knockdown sensitizes several different breast cancer cell lines to paclitaxel, doxorubicin, cisplatin, 4-hydroxycyclophosphamide, hydrogen peroxide, and UV-radiation. The chemoresistance function of MTDH/AEG-1 has also been extended to neuroblastoma (30) and prostate cancer.3 MTDH/AEG-1 does not affect the uptake or retention of chemotherapy drugs. Instead, MTDH/AEG-1 may increase

3 G. Hu et al., unpublished results.
Chemoresistance by promoting cell survival after chemotherapeutic stress. This could be mediated by the prosurvival pathways such as PI3K and NfκB, or through other downstream genes of MTDH/AEG-1 that directly regulate chemoresistance. Microarray analysis of breast cancer cells revealed that MTDH/AEG-1 knockdown led to decreased expression of chemoresistance genes ALDH3A1, MET, HSP90, and HMox1, and increased expression of pro-apoptotic genes BNIP3 and TRAIL (29). Among these genes, ALDH3A1 and MET were validated to partially contribute to the chemoresistance role of MTDH/AEG-1 in MDA-MB-231 breast cancer cells (29). Microarray analysis of MTDH/AEG-1 overexpression in HepG2 cells reveal the panel of genes that may also contribute to chemoresistance. These genes included drug-metabolizing enzymes for different chemotherapeutic agents, such as dihydropyrimidine dehydrogenase (DPYD), cytochrome P4502B6 (CYP2B6), dihydrodiol dehydrogenase (AKR1C2), and the ATP-binding cassette transporter ABCG11 for drug efflux (28). Together, these genes may mediate the broad-spectrum chemoresistance function of MTDH/AEG-1 in different cancer types.

**Clinical-Translational Advances**

Consistent with the role of MTDH/AEG-1 in many different aspects of tumor malignancy, recent clinical studies have convincingly linked MTDH/AEG-1 with tumor progression and poor clinical outcomes in many cancer types, including breast cancer, prostate cancers, glioma, esophageal cancer, and hepatocellular carcinoma. These findings suggest that MTDH/AEG-1 may be developed as a powerful independent poor-prognosis marker and a molecular target for anticancer therapeutics.

**Breast cancer.** MTDH/AEG-1 is expressed in low levels or is absent in most of normal human breast tissues, but was found to be frequently overexpressed in breast cancer cell lines or breast tumors (7, 8, 29, 31). Two independent analyses using breast tumor samples collected in the United States and in China revealed strikingly similar patterns of MTDH/AEG-1 expression and clinical association (29, 31). MTDH/AEG-1 is abundantly expressed in about 44 to 47% of the primary tumors and is significantly correlated with clinical stage, tumor size, lymph node spread, distant metastasis, and poor survival (29, 31). MTDH/AEG-1 expression was not correlated with other common clinicopathological parameters including age, estrogen receptor, progesterone receptor, HER2, and p53 status. No significant difference of MTDH/AEG-1 expression was observed in basal or luminal subtypes of breast tumors (29). Multivariate analysis suggested that MTDH/AEG-1 expression is an independent prognostic indicator for the survival of patients with breast cancer (29, 31). MTDH/AEG-1 is located at chromosome 8q22, a region frequently amplified in many cancers and is associated with poor prognosis (29, 32–35). Indeed, MTDH/AEG-1 is consistently found to be overexpressed in breast tumors with genomic gain of 8q22 (29), although a substantial fraction of tumors with normal copies of MTDH/AEG-1 also overexpress the protein, suggesting alternative mechanisms of MTDH/AEG-1 up-regulation (e.g., through c-Myc activation). Genomic gain of 8q22 has also been associated with increased expression of MTDH/AEG-1 in glioma and liver cancers (7, 28).

**Esophageal squamous cell carcinoma.** Similar to the breast cancer studies, immunohistochemical analysis of 168 esophageal squamous cell carcinoma (ESCC) specimens revealed that 47.6% of tumors exhibited high levels of MTDH/AEG-1 expression (36). Overexpression of MTDH/AEG-1 was significantly correlated with the clinical stage and various tumor grading parameters, as well as shorter survival. Multivariate analysis again indicated that MTDH/AEG-1 expression as an independent poor-prognostic indicator for ESCC patients (36).

**Prostate cancer.** MTDH/AEG-1 is overexpressed in prostate cancer samples and cell lines compared with benign prostatic hyperplasia tissue samples and normal prostate epithelial cells (11, 22). MTDH/AEG-1 inhibition reduces cell viability and promotes apoptosis of prostate cancer cells, but not normal prostate epithelial cells (22). MTDH/AEG-1 is also shown to affect the invasive property of PC3 and DU145 prostate cancer cells (22). Interestingly, decreased nuclear staining of MTDH/AEG-1 was associated with increased Gleason grade and shorter survival of patients (11). MTDH may have a nuclear function in normal prostate tissue and is lost in tumorigenesis.

**Hepatocellular carcinoma.** In hepatocellular carcinoma cells, expression of MTDH/AEG1 gradually increases from stage I to IV and with a decreasing degree of differentiation (28). MTDH/AEG-1 overexpression enhances anchorage-independent growth, matrigel invasion, in vivo tumorigenicity, and angiogenesis through the enhancement of PI3K/AKT, MAPK, and Wnt/β-catenin pathways (28).

MTDH/AEG-1 overexpression has also been documented in glioma, melanoma, and neuroblastoma (7, 30). Overall, MTDH/AEG-1 overexpression is strongly correlated with advanced tumor characteristics and poor clinical outcomes and is a promising target for novel therapeutics.

**Clinical translation and therapeutic targeting strategy.** First of all, MTDH/AEG-1 overexpression or genomic amplification can be used as biomarker to identify subgroups of patients who require more aggressive treatment and are likely to benefit from MTDH/AEG-1 targeted therapies. Patients with MTDH/AEG-1 overexpression or amplification in their tumors are more likely to suffer from metastatic recurrence and may need to be monitored closely for clinical signs of relapse so that therapeutic interventions can be applied early enough for optimal outcomes. Furthermore, for these high-risk patients, a higher dose of chemotherapy may be required and combination of chemotherapy with MTDH/AEG-1 inhibition may help increase the efficacy of chemotherapy. There are several possible avenues to develop novel cancer treatments on the basis of molecular targeting of MTDH/AEG-1. Neutralizing antibodies against MTDH/AEG-1 can be used to block its function in endothelial adhesion and reduce the risk of metastasis (8). Polyclonal antibodies against the LHD of MTDH/AEG-1 has been shown to reduce lung metastasis by 40% when co-injected with 4T1 mouse mammary tumor cells in experimental lung metastasis assays (8). Similar experiments using different treatment windows in preclinical metastasis models of human breast cancer need to be tested to further validate the feasibility of this approach before humanized monoclonal antibodies against MTDH/AEG-1 can be developed for clinical trials. Alternatively, siRNA-based reagents
can be developed to reduce MTDH/AEG-1 expression if the high efficiency of siRNA delivery to tumors in vivo can be achieved. Indeed, inhibition of MTDH/AEG-1 expression by RNA interference has been shown to effectively reduce metastasis by three- to tenfold in an MDA-MB-231 xenograft model of breast cancer lung metastasis (29). Furthermore, MTDH/AEG-1 knockdown sensitizes chemoresistant MDA-MB-231 breast tumors to pacli- taxel or doxorubicin (29). In hepatocellular carcinoma, adeno- viral delivery of MTDH/AEG-1-targeting shRNA inhibits xenograft primary tumor growth in mice (28). Thus, MTDH/ AEG-1 inhibition may be applied in neoadjuvant or adjuvant settings to not only increase the response rate of chemotherapy and reduce tumor growth, but also to reduce the systemic spread of metastatic cancer. Finally, identification of functionally important interactions between MTDH/AEG-1 and its partners may lead to the discovery of small molecule compounds targeting MTDH/AEG-1. Because a considerable level of MTDH/ AEG-1 is located in the cytoplasm and nucleus, inhibitors that block its intracellular functions may be needed to effectively re- duce its multiple functions in promoting tumor malignancy. However, as a novel protein with poorly characterized func- tions, it is currently difficult to speculate what kind of small molecule inhibitors can be used to block the intracellular func- tion of MTDH/AEG-1. Further functional characterization of MTDH/AEG-1 is urgently needed to realize its full therapeutic potential.

Conclusions

As a relatively novel gene, MTDH/AEG-1 has emerged as an important regulator in multiple aspects of cancer development and progression. Clinical and functional analyses have estab- lished this multifunctional gene as a potentially valuable target in cancer treatments. However, the functional mechanisms of MTDH/AEG-1 in regulating oncogenic signaling pathways re- main poorly understood and some of the findings need to be validated in a broader collection of model systems. In addition to its direct interaction with p65 and CBP, MTDH/AEG-1 was re- cently found by a yeast hybrid screen to interact and destabilize BCCIPα, a cofactor for tumor suppressors BRCA2 and p21 cip (37). Additional interacting partners for MTDH/AEG-1, particu- larly its adhesion receptor in endothelial cells, still need to be identified. Further studies to clarify the normal physiological roles of MTDH/AEG-1, the function of its various isoforms, as well as the regulation of its cellular localization will facilitate the development of novel cancer treatments through molecular targeting of MTDH/AEG-1.

Disclosure of Potential Conflicts of Interest

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