MTDH and FOXM1, Two Master Regulators in Gynecologic Cancer

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Abstract

Drug resistance and metastasis are the major challenges for treatment of gynecologic cancers. Tumor heterogeneity caused by diverse driver mutations in gynecologic cancers restricts the effective application targeted therapies. Both FOXM1 and MTDH are overexpressed and correlated with drug resistance and metastasis in various types of cancers including gynecologic cancers. Accumulated clinical and functional studies have demonstrated that elevated expression of both FOXM1 and MTDH is the consequence of diverse activating mutations in oncogenes such as PI3K, Ras, myc and loss of function mutations in tumor suppressor genes such as p53 and PTEN. We discuss FOXM1 and MTDH as potential prognostic markers and therapeutic targets in gynecologic cancers.

ABBRiEvATIONS


INTRODUCTION

Although survival rate in gynecologic cancers have improved somewhat in the last decades, there are still many challenges including early diagnosis, prevention, drug resistance, metastasis and drug toxicity [1]. Tumor heterogeneity limits effective application of a standard single treatment modality in gynecologic cancers [2]. These complexities represent major challenges and impediments to developing effective cancer therapies. Newly emerging targeted molecular inhibitors, especially when used in combination with chemotherapy, are promising and may allow us to tailor treatment to individual patient and tumor genetic profiles [2,3]. Currently, mammalian target of rapamycin (mTOR) inhibitors, poly-ADP-ribose polymerase (PARP) inhibitors, tyrosine-kinase inhibitors for vascular endothelial growth factor (VEGF), vascular endothelial growth factor receptors (VEGFR), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and components of the EGFR pathway are being tested in clinical trials for gynecologic cancers [4]. Identification of new molecule(s) that are key effectors of carcinogenesis and progression in many tumor types and which can be targeted therapeutically would be a significant advancement in the development of new treatments. We propose that two such molecules, FOXM1 [5-9] and MTDH [10-13], meet these criteria and can serve as novel diagnostic markers and therapeutic targets in gynecologic cancers.

Overexpression of MTDH and FOXM1 in cancers

Elevated FOXM1 expression has been reported in various types of malignancies including gynecologic cancers. Overexpression of FOXM1 has been observed in 87% of high-grade serous ovarian tumors [14]. FOXM1 has been identified as one of the most commonly upregulated genes in human solid tumors in several gene expression profiling studies. The forkhead box class O (FOXO) family is comprised of multifunctional transcription...
factors (FOXO1, FOXO3a, FOXO4 and FOXO6) involved in fine-tuning a broad repertoire of downstream target genes which impact in cell cycle arrest, invasion, migration, and resistance to therapy [15]. Inactivation and suppression of FOXO, in particular FOXO1 and FOXO3a, have been implicated in tumorigenesis and cancer progression. However, Forkhead subfamily member FOXM1 functions as a classic oncogene [16,17]. FOXO and FOXM1 bind to the same response elements in target genes. Target gene transcription activated by FOXM1 is usually repressed by FOXO. FOXM1 itself is a direct target repressed by FOXO (Figure 1). This repression is released by the inhibition of FOXO proteins via the PI3K-AKT-FoxO axis. Sensitivity to many anti-cancer drugs, including chemotherapy agents paclitaxel, doxorubicin, cisplatin and targeted therapies lapatinib, gefitinib, imatinib, and tamoxifen, are determined through PI3K-AKT and downstream FOXO –FOXM1 axis [22-26].

An inverse relationship between MTDH expression level and OS (overall survival), PFS (progression free survival) and metastasis free survival has been observed in a large number of studies in diverse cancers [27-29]. MTDH overexpressions was detected in more than 40% of breast cancer patients compared to normal tissues and was strongly correlated with poor outcome and metastasis in more than 1000 breast cancer patients. Genomic amplification is one of the mechanisms for the increased MTDH expression in breast cancer [30]. For the most lethal gynecological cancer in Western countries, epithelial ovarian cancer (EOC), high MTDH expression was detected in 64.8% with peritoneal metastasis and 83.7% with lymph node metastasis among 157 patients with EOC, including 49 patients with lymph node metastasis and 128 patients with peritoneal dissemination [31,32]. For the most common type of ovarian cancer, ovarian serous carcinoma, expression of MTDH was significantly higher in patients with stage II–IV tumors which were resistant to cisplatin in sensitive patients [33-35]. Median PFS and OS were 30.4 months and 35.28 months, respectively, in the high MTDH expression group versus 63.6 months and >50 months in the low MTDH expression group (p<0.001). Thus, increased expression of MTDH predicts for poor response to cisplatin and shorter survival. Correlation of MTDH expression with surgical debulking has been confirmed in a systemic review of 279 patients with stage 3 or 4 serous EOC in the Cancer Genome Atlas ovarian cancer data set. In endometrial cancer, the most common cancer of the female genital tract, overall 5-year survival rate is more than 80% if disease is diagnosed early. However, a substantial percentage of patients will relapse and develop metastatic disease. A biomarker to identify high risk patients up front needs to be developed. We propose that MTDH may serve this purpose. MTDH expression is increased.

Molecular mechanisms of increased expression FOXM1 and MTDH in cancer

There are six mechanisms through which FOXM1 expression is elevated in different types of cancers. These are: (i) amplification of the FOXM1 gene locus. The FOXM1 locus is located at chromosome 12p13, a region frequently amplified in non-Hodgkin’s lymphoma (NHL), cervical carcinomas and breast adenocarcinomas [36,37] (ii) Increased protein stability. FOXM1 stability in cancer cells is increased via the Wnt signaling pathway [38] direct interaction with nucleophosmin [39] and phosphorylation by Cdk4,6/cyclinD complexes (Figure 1) [40] (iii) Increased transcription. Transcription of FOXM1 is activated through the action of transcription factors E2F, c-Myc, and hypoxia-inducible factor-1 (HIF-1) [41-43]. (iv) Mutations in the tumor suppressor genes p53 and Rb. FOXM1 is regulated by two major tumor suppressors Rb and p53. Transcription of FOXM1 is repressed by p53 via a direct interaction on the p53 response element of the FoxM1 promoter. Inactivation of p53 releases FOXM1 transcriptional repression in cancers [44-46]. Rb inhibits FOXM1-mediated downstream transcription by directly interacting with FOXM1. Phosphorylation of Rb by Cyclin D1/Cdk4 disrupts the repression of FOXM1 by Rb [47] (v) Activation by oncogenic signaling pathways. FOXM1 is activated by oncogenic signaling pathways including PI3K/Akt, EGFR, Raf/MEK/ERK, and Hedgehog [48]. (vi) Reduced expression of FOXM1 targeting microRNAs results in the reduction of FOXM1 in cancer cells. Five miRNAs, miR-370, miR-31, miR-34, miR-134 and miR-200b, have been shown to reduce FOXM1 expression at the mRNA or protein level [49-53].

MTDH is also regulated in cancer by a variety of mechanisms. (i) MTDH overexpression results from amplification of the MTDH locus at chromosome 8q22 in breast tumors [30] (ii) Activation of H-ras markedly induces expression of MTDH through the PI3K/AKT signaling pathway by increasing the association of c-Myc to the E-box elements of the MTDH promoter (Figure 1) [21,54] (iii) Induction of MTDH expression by hypoxia and glucose deprivation. Stabilization of HIF-1α in response to activation of the PI3K/AKT pathway results in induction of MTDH in glioma cells [55]. The increased expression of MTDH which is associated with glucose deprivation is dependent on the production of reactive oxygen species (ROS); in turn, increased MTDH inhibits ROS production [56] (iv) LPS (lipopolysaccharides) can induce MTDH via activation of the NF-κB pathway in human promonocytic cells and in breast cancer cells. MTDH is required for LPS-induced NF-
kB activation via a positive feedback loop between MTDH and NF-kB. MTDH mediated IL-8 and MMP-9 expression is critical in LPS-induced invasion and metastasis [57,58]. Reduced expression of tumor suppressor miRNAs such as miR375 and mir26a, which target MTDH, result in increased MTDH levels in cancer cells [59-61]. MTDH is a direct target of three miRNAs, miR-26a, miR-375 and miR-136. MiR-26a represses MTDH expression by directly targeting the 3’UTR of MTDH mRNA in breast cancer cells [61]. MiR-375 is a well-known tumor suppressor and has been shown to downregulate the expression of MTDH by binding to its 3’-UTR [59,60]. Reverse expression patterns of decreased miR-375 and increased MTDH have been observed in several tumor types including including Head and Neck Squamous Cell Carcinoma (HNSCC), Esophageal Squamous Cell Carcinoma (ESCC), liver cancer and breast cancer [56,57]. MTDH was also identified as a target of miR-136 in glioma cells [62].

Role of FOXM1 and MTDH in drug resistance

The role of FOXM1 in DNA repair, which occurs by homologous recombination (HR) but not non-homologous end joining (NHEJ), has been demonstrated using an integrated direct repeat green fluorescent protein (GFP) reporter system of DSB (double strand break) repair in HeLa cells. Two critical proteins for HR repair, BRIP1 (BRCA1-associated BACH1 helicase) [63] and Rad51, [64] have been identified as direct transcriptional targets of FOXM1 by promoter analysis and chromatin-immunoprecipitation. Correlation of FOXM1 with the expression of two other DNA repair genes, XRCC1 (X-ray repair cross-complementing protein 1) and BRCA2 (breast cancer-associated gene 2), has been observed in osteosarcoma cells [65]. The occurrence of genomic instability in cancer cells with FOXM1 overexpression has also been shown to be linked to loss of heterozygosity and copy number variations (CNV) measurement. The role of FOXM1 in the DNA damage response and in DSB HR repair implicates it as an attractive target for therapies employing DNA damaging agents such as platinum, topoisomerase inhibitors, IR, or alkylators. Indeed, targeting FOXM1 increases the sensitivity of tumor cells to DNA damaging agents [66-68).

A transgenic mouse with hepatocyte-specific expression of MTDH (Alb/MTDH) accelerated development of HCC when exposed to the hepatocarcinogen, N-nitrosodiethylamine (DEN) compared to wild type mouse [69]. Alb/AEG-1 hepatocytes display significant resistance to senescence. Reduced ROS levels and fewer senescent cells as well as activation of ATM, ATR, CHK1, and CHK2 occurs in Alb/AEG-1 hepatocytes following isolation compared to control hepatocytes. This indicates that MTDH is involved in the DDR response. A further development has been the identification of MTDH as a new RNA binding protein with the potential to control the levels of important DNA repair factors at the post-transcriptional level. Association of MTDH with various miRNAs was recently identified by RNA-binding protein immunoprecipitation followed by microarray analysis ( RIP-chip) [18]. The miRNAs bound to MTDH encode various functional proteins including multiple members of the Fanconi pathway (FANCA, FANCD2, FANCJ) [18,70]. An attractive hypothesis is that high expression of MTDH allows cancer cells to rapidly repair DNA in the setting of cell stress, including chemotherapy, and this potentiates therapeutic resistance.

Therapeutic opportunities of targeting FOXM1 and MTDH

There are a number of ways in which FOXM1 and MTDH can be targeted. Thiazole compounds, Siomycin A and thiostrepton, have been identified as potential FOXM1 small chemical inhibitors [71,72]. Both Siomycin A and thiostrepton can repress the transcriptional activity of FOXM1 and decrease FOXM1 at the mRNA and protein level in cancer cells. The action of both Siomycin A and thiostrepton could be related to their ability to function as proteasome inhibitors [73]. Neither agent causes any anti-proliferation or apoptotic effects on untransformed cells, thus making them potentially attractive therapeutic drugs for cancers with FOXM1 overexpression. FOXM1 is regulated by NPM1 and ARF [74,75]. NPM promotes cancer cell survival and FOXM1 is a novel inhibitory target of p19 (ARF). A p19 (ARF) 26-44 peptide containing nine D-Arg residues was sufficient to reduce FOXM1 transcriptional activity and tumor proliferation in HCC. Interaction of FOXM1 with the multifunctional protein NPM has been observed by mass spectrometry analysis, co-immunoprecipitation and glutathione S-transferase pull-down. Knockdown of NPM caused significant down-regulation of FOXM1 in cancer cells to the levels found in normal cells. The interaction between FOXM1 and NPM could potentially be disrupted by peptides or small molecules, leading to a FOXM1 targeted therapy. A potential future therapeutic strategy might be to delivery RNA molecules which target MTDH or FOXM1; however, employing RNA-based regimens for treatment will require significantly more research and development before they can be widely used in patients. The potential anticancer agents ursolic acid [76,77] and cryptotanshinone [78] have been found to repress expression of MTDH in ovarian and prostate cancer. HIF-1α was shown to be involved in the repression of MTDH by cryptotanshinone [78]. In addition, cadmium chloride reduced MTDH expression and NF-kB activity in breast cancer cells [79].

CONCLUSION AND FUTURE PERSPECTIVES

In summary, both MTDH and FOXM1 are emerging as critical master regulators of cancer development that may affect all of the hallmarks of cancer. Both FOXM1 and MTDH might become markers routinely used in a clinical diagnostic laboratory as well as therapeutic targets to overcome drug resistance in diverse cancers. The presence of increased MTDH on the surface of cancer cells might be developed as antibody-based diagnostic or therapeutic [80-82]. Further work to understand the function of MTDH is warranted. The following questions must be investigated: 1. What is the biological significance of MTDH in normal physiological conditions? 2. Do any of the different isoforms/modifications of MTDH identified from sequence predictions contribute to the impact of MTDH in cancer? MTDH has a broad range of protein and RNA binding partners in different cellular components. 3. Does MTDH function as a scaffold protein or does it directly influence the function or expression of its different binding partners? Understanding these basic cellular and biochemical properties of MTDH will help in the development of effective inhibitors. For FOXM1, more studies are needed to develop more specific inhibitors of FOXM1 to achieve clinical benefits. Oncogenic miRNAs regulated by FOXM1 could be developed as new therapeutic targets. In addition, tumor
suppressor miRNAs that repress FOXM1 could be developed as FOXM1 inhibitors.

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