Metadherin is an apoptotic modulator in prostate cancer through miR-342-3p regulation

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Abstract

Prostate cancer is the second most common cancer in men worldwide. This study focused to clarify the roles of Metadherin (MTDH) and miR-342-3p in prostate cancer. We identified that MTDH was up-regulated and miR-342-3p was down-regulated in the prostate tissues, and there is an inverse correlation between MTDH and miR-342-3p. Functional studies revealed that miR-342-3p directly targets MTDH via binding to the 3' untranslated regions (UTRs) in the prostate cancer cells. Moreover, we also found MTDH overexpression in DU145 and PC3 cells inhibited apoptosis. Subsequently, miR-342-3p has been revealed to reverse the MTDH effect on the cellular apoptosis in the further studies. Our results indicate that MTDH repress apoptosis of prostate cancer in vitro and provides a new strategy for human prostate cancer therapy in the future.

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1. Introduction

Prostate cancer is the second highest cause of cancer mortality after lung tumors (El-Shami et al., 2015; Castillejos-Molina and Gabilondo-Navarro, 2016). The incidence rates of prostate cancer have been remarkably increased in China which partly is resulted from the gradual increase of the aging population and wide use of screening test. At present, there is not a clear, proven effective therapy for prostate cancer. Therefore, great efforts have been made to facilitate the discovery of new therapeutic targets for prostate cancer.

Metadherin (MTDH), also known as astrocyte elevated gene-1 (AEG-1) and lysine-rich CEACAM1 coisolated (LYRIC), was originally cloned as a human immunodeficiency virus-1 (HIV-1)-inducible gene in primary human fetal astrocytes (Su et al., 2002). MTDH has emerged as an import oncogene that is overexpressed in various cancers (Hu et al., 2009a, Li et al., 2016; Suzuki et al., 2017; Zhang et al., 2017). MTDH has been found to promote metastasis by supporting putative cancer stem cell properties and epithelial plasticity in pancreatic cancer (Suzuki et al., 2017). MTDH has also been reported to promote glioma invasion through regulating miR-130b-ceRNAs (Tong et al., 2017). However, the regulatory network of MTDH is not fully understood in prostate carcinoma.

miRNAs function as single strand RNA molecules of about 22nt in length and repress the expression of genes through partial binding to the 3' UTRs of even hundreds of target genes (Osada and Takahashi, 2007; Kumar et al., 2008). More and more investigation has reported that miRNAs contribute to the cancer progression (Lewis et al., 2003; Szabo and Bala, 2013). MiR-342-3p has been found to regulate MYC transcriptional activity via direct repression of E2F1 in human lung cancer (Tai et al., 2015). MiR-342-3p has also been observed to target RAP2B to suppress proliferation and invasion of non-small cell lung cancer cells (Xie et al., 2015). Although much is known about the biological functions of miR-342-3p in many tumors and tissues, the function of miR-342-3p in prostate cancers has not yet been completely elucidated.

Very little is known regarding the role of MTDH and miR-342-3p in the progression of prostate cancer. In addition, the potential mechanisms regulating MTDH expression via miR-342-3p is poorly...
understood. In this study, we identified an inverse correlation between MTDH and miR-342-3p in prostate tumor tissues, and MTDH is repressed by miR-342-3p in vitro. Further investigation revealed that MTDH suppressed the apoptosis, and miR-342-3p reversed the MTDH effect on the apoptosis of prostate cancer cells. Moreover, MTDH regulates the expression of pro- and anti-apoptotic factors through miR-342-3p in vitro. Our studies suggest that MTDH is an apoptotic modulator in prostate cancer through miR-342-3p regulation, and MTDH may be an unexplored target of a novel therapy for the prostate cancer.

2. Materials and methods

2.1. Cell culture and tumor tissues

The human prostate cancer cell lines DU145 and PC3 were purchased from ATCC (Manassas, VA) and maintained in the medium according to the manufacturer (RPMI-1640 (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin).

For tumor tissues, tissues were frozen in liquid nitrogen immediately after minced on ice and stored at –80 °C until analyze.

2.2. Quantitative real-time PCR (qRT-PCR)

Total RNA from tissue samples and cells were isolated using the mirVanaTM miRNA isolation kit (Ambion, Austin, TX). After removal of the residual DNA by DNase I (Invitrogen), RNAs were converted into cDNA by the high capacity RNA-to-cDNA kit (Thermo Fisher Scientific, Waltham, MA). The specific gene expression was determined by Taqman qRT-PCR and performed on a QuantStudio 6 Flex system (Life technologies, Gaithersburg, MD). Gene expression was normalized to GAPDH.

For the mature miR-342-3p quantification, total RNA was reverse-transcribed using the Taqman advanced miRNA cDNA synthesis kit (Applied Biosystems). U6 small nuclear RNA (snRNA) was used as the internal control and reverse-transcribed by Taqman microRNA reverse transcription kit following manufacturer’s protocol (Applied Biosystem). U6 was used as an internal control.

All the specific primers for gene and miRNA expression are purchased from Applied Biosystem. The amount of each gene or miRNA relative to the internal control was calculated as previously described (Livak and Schmittgen, 2001).

2.3. Transfection of miR-342-3p mimic

miR-342-3p mimic was commercially available from Thermo-Fisher Scientific (Foster City, CA). Transfection of miR-342-3p mimic was performed by the RNAiMAX Reagent (Invitrogen) according to the manufacturer. miRNA vector control (miR-NC) was also purchased from Thermo Fisher Scientific.

2.4. Prediction of miR-342-3p targets

To decipher the relationship between miR-342-3p and MTDH, we used two public databases including TargetScan and RNA22 v2) to predict candidate miRNA targeting MTDH.

2.5. 3'-UTR dual luciferase assay

The DU145 and PC3 cells were seeded in a 6-well plate (BD Biosciences, Bedford, MA) at a density of 0.25 M cells/well with the complete media. The cells were co-transfected with 1ug of MTDH 3'-UTR luciferase reporter construct with 20 nM miR-342-3p mimic or miR-NC with lipofectamine 2000 (Invitrogen) with Opti-MEN (Thermo fisher scientific). The cells were lysed and subjected to luciferase assay using dual-luciferase reporter assay reagent (GeneCopoeia, Rockville, MD) after 48 h according to the manufacturer’s instruction. The 3'-UTR of MTDH was commercially available from GeneCopoeia.

2.6. MTDH overexpression and silencing

Lentiviral system (Applied Biological Materials, Canada) to stably overexpress MTDH (Lenti-MTDH) and its negative control vector (Lenti-VC) in the cells followed the manufacturer’s instruction. The cells, 2 × 10⁶ cells per well on a 6-well plate, were transiently transfected with MTDH small interfering siRNA (si-MTDH) or control siRNA (si-Ctrl) (Santa Cruz Biotechnology, Santa Cruz, CA) following the manufacturer’s protocol. Cells were incubated for 5 to 7 h at 37 °C in a 5% CO₂ incubator with the siRNA transfection medium (Santa Cruz Biotechnology). After then, the medium was replaced by the complete medium. The efficiency of transfection was determined after 3 days.

2.7. Western blotting analysis

The total protein lysis of treated or untreated cells (15µg) was separated on 4–15% precast gels (Bio-Rad, Richmond, CA). After then, the separate proteins were blotted onto nitrocellulose membranes (Bio-Rad, Richmond, CA). Membranes were blocked with 5% dried non-fat milk powder in TBST for 1 h at room temperature, then incubated with primary antibodies overnight at 4°C. After being washed with TBST 3 times, the membranes were incubated with the corresponded secondary antibody (Bio-Rad) for 2 h at room temperature. The band signals of target proteins were visualized using the BeyoECL Plus Detection System (Beyotime Institute of Biotechnology) and the images were obtained by X-ray film exposure. β-Actin was used as normalization of protein loading.

Antibody against MTDH and β-Actin was purchased from Cell Signaling Technologies (Beverly, MA) and Sigma-Aldrich (St Louis, MO).

2.8. Flow cytometric analysis for apoptosis

The cell viability was compared against the MTDH overexpression or miR-342-3p overexpression and their correlated control counterparts. The annexin V-FITC kit was used to detect cells undergoing apoptosis according to the manufacturer’s protocol (BD Biosciences, San Jose, CA). Propidium iodide (PI) is widely used in conjunction with Annexin V to determine if cells are viable, apoptotic, or necrotic through differences in plasma membrane integrity and permeability.

2.9. Statistics

Results are shown as means ± SE. Student’s 2 pair t-test was used to compare the significance of differences between the mean of different groups. SPSS (SPSS Inc. Chicago, IL) was used to calculate the significance and a value of P < 0.05 considered as the statistical significance. The correlation between MTDH and miR-342-3p was assessed by the x² test. Pearson r, correlation coefficient represented a semi-quantitative measure of linear association among two parameters with r > ±0.5 considered a strong relationship.
3. Results

3.1. An inverse correlation between MTDH and miR-342-3p in tumor tissues

Of the tumor tissues (13 cases) and noncancerous prostate tissues (16 cases), MTDH mRNA expression was found to be significantly up-regulated in the prostate tumor tissues compared with the noncancerous prostate tissues (Fig. 1A). MiR-342-3p expression was observed to down-regulated in the prostate tumor tissues compared with the noncancerous prostate tissues (Fig. 1B). We next examined the correlation between the expression of MTDH and miR-342-3p using a linear regression analysis. A significant inverse correlation was found between the expression of miR-342-3p and MTDH (Fig. 1C).

3.2. MTDH is repressed by miR-342-3p

To verify whether MTDH is the potential target of miR-342-3p, DU145 and PC3 cells were cotransfected with MTDH 3'-UTR reporter and miR-342-3p or miR-NC. We observed that miR-342-3p significantly reduced the luciferase activity of MTDH 3'-UTR reporter compared with the miR-NC (Fig. 2A). Next, we confirmed the MTDH repression through miR-342-3p by western blotting. As shown in Fig. 2B, MTDH protein expression was reduced by miR-342-3p overexpression. This data suggests that miR-342-3p is involved in the regulation of MTDH.

3.3. MTDH suppresses the apoptosis in prostate cancer cells

To assess the effects of MTDH on the biological behavior of prostate cancer cells in vitro, we stably overexpressed MTDH in the DU145 and PC3 cells (Fig. 3A). The apoptosis of DU145 and PC3 cells responding to MTDH overexpression was assessed by Annexin V. We found significant decreases of apoptosis in cells overexpressing MTDH (P < 0.05) compared to the parent cells (Fig. 3B). Subsequently, we silenced the MTDH with si-MTDH (Fig. 3C). We found the loss of MTDH significantly increased the apoptosis in the DU145 and PC3 cells versus the parent cell lines (Fig. 3D), which reversed the effect of MTDH overexpression on prostate cancer cells.

3.4. miR-342-3p blocks the effect of MTDH on the apoptosis of prostate cancer cells

To assess whether miR-342-3p contributes to the effect of MTDH on the apoptosis in the DU145 and PC3 cells, miR-342-3p was transiently up-regulated by miR-342-3p mimic in the cells...
overexpressing MTDH (Fig. 4A). We detected that miR-342-3p overexpression blocked the effect of MTDH overexpression on the apoptosis in the cells (Fig. 4B). This data suggests that miR-342-3p contributes to the MTDH regulatory network to the apoptosis in prostate cancer cells.

3.5. MTDH regulates the expression of pro- and anti-apoptotic factors through miR-342-3p

To unravel the relevant molecular mechanism underlying MTDH contributed to the apoptosis of prostate cancer cells, we next asked whether MTDH played important roles on the expression of pro- and anti-apoptosis factors (Bax, Bad, Bcl-2 and Mcl-1). As shown in Fig. 5A, MTDH overexpression significantly up-regulates the mRNA levels of anti-apoptotic factors, Bcl-2 and Mcl-1, but down-regulates the mRNA levels of pro-apoptotic factors, Bad and Bax, in both PC-3 and DU145 cells. Subsequently, we found miR-342-3p overexpression reversed the effects of MTDH on those apoptotic factors in both PC-3 and DU145 cells (Fig. 5B). miR-342-3p overexpression remarkably down-regulates the mRNA levels of anti-apoptotic factors, Bcl-2 and Mcl-1, but up-regulates the mRNA levels of pro-apoptotic factors, Bad and Bax. This data suggests that MTDH regulates the apoptosis in prostate cancer cells through miR-342-3p.

4. Discussion

While the normal physiologic role of MTDH remains elusive (Yoo et al., 2011), we identify that the prostate cancer is another tumor type with elevated MTDH. To the best of our knowledge, this is the first report to link MTDH and miR-342-3p in the prostate cancer.

The roles of MTDH have been established in tumor progression, including transformation, apoptosis, invasion, metastasis and chemoresistance (Hu et al., 2009a, 2009b; Liang et al., 2015; Shi and Wang, 2015). It has been reported that the FBXW7 tumor suppressor inhibited breast cancer proliferation and promotes apoptosis by targeting MTDH for degradation (Chen et al., 2018a,b). And, MTDH-SND1 interaction is crucial for expansion and activity of tumor-initiating cells in diverse oncogene- and carcinogen-induced mammary tumors (Wan et al., 2014). Additionally, huaier granule extract inhibit the proliferation and metastasis of lung cancer cells through down-regulation of MTDH, JAK2/STAT3 and MAPK signaling pathways (Chen et al., 2018a,b). However, the role of MTDH in prostate cancer has not been investigated in detail. In this study, MTDH also can affect the cellular apoptosis in prostate cancer. MTDH was found to be up-regulated in the prostate tumor tissues compared with the non-cancerous tissues. In order to clarify the biological functions of MTDH of prostate cancer, we conducted this study at the cellular level. Overexpression of MTDH suppresses the apoptosis, while silencing of MTDH reverse the effect on apoptosis caused by MTDH overexpression in prostate cancer cells. Taken together, these data indicate that MTDH is associated with the progression of prostate cancer.

MiRNAs are well-known as noncoding RNAs that regulate gene expression by posttranscriptional silencing of coding genes. MiRNA dysregulation in prostate and other type of cancers implicates their roles in cancer biology. Moreover, the functional study of miRNAs provide support for the new knowledge in biological processes of cancer. The deregulation of several miRNAs has been found to function as tumor suppressors or oncogenes, such as miR-34a (Liu et al., 2011), miR-21 (Li et al., 2009; Ribas et al., 2009) and miR-26b (Li et al., 2013). MiR-342-3p has been shown to suppress proliferation, migration and invasion by targeting FOXM1 in human cervical cancer (Li et al., 2014). And, it also has been reported that miR-342-3p suppresses hepatocellular carcinoma proliferation through inhibition of IGF-1R-mediated Warburg effect (Li et al., 2018). In this study, to identify the miRNA specific for prostate cancer, we found that miR-342-3p is down-regulated in the prostate tumor tissues. Moreover, miR-342-3p has also been found to be inversely correlated with MTDH in prostate tissues. In addition, transfection of synthetic miR-342-3p remarkably reduced the MTDH protein level. Furthermore, miR-342-3p blocks the effect of MTDH on the apoptosis of prostate cancer cells. Therefore, it is reasonable to believe that miR-342-3p plays a regulatory role of MTDH in prostate cancer.

In summary, we identified the biological function of MTDH which is overexpressed in prostate tumor tissues. MTDH can regulate the apoptosis of prostate cancer through miR-342-3p. It provides a potential therapeutic strategy for prostate cancer.
Fig. 3. MTDH overexpression suppresses the apoptosis in prostate cancer cells. (A) Taqman qRT-PCR analysis and western blotting were performed to determine MTDH expression after MTDH overexpression. (B) The annexin V-FITC kit was used to detect cells undergoing apoptosis after MTDH overexpression. (C) Taqman qRT-PCR analysis and western blotting were performed to determine MTDH expression after MTDH silencing. (D) The annexin V-FITC kit was used to detect cells undergoing apoptosis after loss of MTDH. β-Actin was used as an internal control. Results are mean ± SE (n = 3). *p < 0.05 vs parent cells.
Fig. 4. miR-342-3p reversed the effect of MTDH on the apoptosis of PC-3 and DU145 cells. (A) Taqman qRT-PCR analysis was performed to determine miR-342-3p expression after miR-342-3p overexpression. U6 was used as an internal control. (B) The annexin V-FITC kit was used to detect cells undergoing apoptosis after miR-342-3p overexpression. Results are mean ± SE (n = 3), *p < 0.05 vs parent cells.

Fig. 5. mRNA expression analysis of pro-apoptotic (Bad and Bax) or anti-apoptotic (Bcl-2 and Mcl1) factors by taqman qRT-PCR in the PC-3 (A) and DU145 (B) cells. β-Actin was used as an internal control. The results were presented as mean ± SE (n = 3). *p < 0.05 vs parent cells.
