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miR-331-3p, miR-338-3p, hsa-let-7b-3p, and HSF2 regulate MSRB3 expression and ROS-mediated apoptosis in cervical cancer; insights from bioinformatics study

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A B S T R A C T

Cervical cancer is one of the most common cancers in women and is the third leading cause of cancer-related mortality. Metabolic dysregulation is a common hallmark of cancer. Reactive oxygen species (ROS) are oxygen metabolism byproducts that are impacted by metabolic instability. ROS can trigger cellular apoptosis as a safety switch by controlling the intrinsic mitochondrial apoptosis pathway. MSRB3(methionine sulfoxide reductase B3) can inhibit ROS-mediated apoptosis in cervical cancer cells, promoting tumorigenesis and metastasis. In this article, the effect of miR-331-3p, and miR-338-3p on reducing gene expression is investigated. The unfavorable proteins in cervical cancer tissues were selected and obtained using the Human Protein Atlas (HPA) database. OncoDB was used to find differentially expressed genes (DEGs) linked with the cancer stage. A Venn diagram was created to identify genes that are both clinically unfavorable and linked with the cancer stage. STRING database was used to predict functional interactions of human proteins with specific genes. The upstream transcription factor, Gene Ontology analysis, and potential mechanism of MSRB3 and its interacting proteins were all investigated using Enrichr. HPA identified 223 genes linked with poor prognosis in cervical cancer patients. OncoDB was used to identify the top 1,000 DEGs related to cervical cancer patients' clinical stages. MSRB3 expression was shown to be correlated with cervical cancer patient survival and clinical stage. A tight relationship between MSRB3 expression and ROS was discovered by Reactome. According to STRING, MSRB3 interacts with two additional genes (TXN(Thioredoxin) and FXN(Frataxin)). According to GO analysis, these genes were engaged in protein repair pathways and had oxidoreductase activity. We discovered that MSRB3 expression is associated with a poor prognosis and survival in cervical cancer patients. Using the miRWalk database, we predicted that the miR-331-3p, miR-338-3p, and hsa-let-7b-3p have the potential to be involved in regulating MSRB3 Also, due to the TRRUST analysis, HSF2 is an upstream transcription factor of MSRB3, TXN, and FXN can be knocked down in order to suppress MSRB3 with two additional genes. Thus, treatment strategies that target MSRB3 expression may prone cervical cancer cells to apoptosis.

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Introduction

Cervical cancer is the most common gynecological malignancy and in 2020, with 604,000 new cases worldwide, with approximately 342,000 related deaths, and is the fourth leading cause of cancer death in women worldwide (Sung et al., 2021). Cervical cancer has become the most common cancer in 23 countries and the leading cause of cancer-related deaths in 36 countries (Bray et al., 2018). Usually, it affects women between the ages of 35 and 44 years old with the average age at diagnosis being 50 years old. It rarely develops in women younger than 20 years old. More than 20% of cases of cervical cancer are found in women over 65 years old (Fontham, 2020; Howlader N, 2019).

Treatment of invasive cancer involves the management of the primary lesion and sites of metastatic disease. Although definitive surgery is typically reserved for patients with stages I or early IIa disease, both surgery and radiation therapy may be used as primary treatments. The ability of radiotherapy to cure locally advanced cervical cancer is limited by the size of the tumor because the doses required to treat large tumors exceed the limit of toxicity in normal tissue (Rose, 1999). Moreover, radiotherapy could be used for local diseases while chemotherapy is used for systemic diseases (Rose, 1999). However, there is a need for advancement in the treatment of locally advanced diseases. Resistance of cancer to radiotherapy and chemotherapy is a primary cause of treatment failure (Saxena et al., 2005). Chemotherapy and radiation therapy are invasive, non-specific, and ineffective. In addition, they leave several side effects in patients. Studies on drug targeting and biomarkers for cervical cancer to date have mainly focused on the role of protein-coding genes, whereas our knowledge of the process of non-functioning gene sequences, such as microRNAs (miRNAs), is still in its infancy. miRNAs are short regulatory RNAs that control gene expression by inhibiting translation by binding to the complementary sites of their target gene transcripts(Bartel, 2004). In addition, molecular biology analysis by informatic tools of HPV-related tumors has identified molecular targets for optimized therapy(Hua, 2017a; Wang et al., 2022).

Reactive oxygen species (ROS) play an essential role in apoptosis, a programmed cell death, by regulating the intrinsic mitochondrial apoptosis pathway(Redza-Dutordoir & Averill-Bates, 2016). Apoptosis regulation is crucial for maintaining normal cellular homeostasis (Redza-Dutordoir & Averill-Bates, 2016). The oxidation of methionine residues in proteins occurs during oxidative stress and can lead to an alteration in protein function. The enzyme methionine sulfoxide reductase (MSR) reverses this modification. MSRB3 acts as an oxidase suggesting a role for methionine oxidation in the regulation of ER protein function. MSRB3 knockdown induces cancer cell apoptosis through endoplasmic reticulum stress-dependent pathways (Cao et al., 2018; Jiang & Moskovitz, 2018).

In the process of tumor progression, genetic alterations can be used to suppress tumorigenesis. Genetic expression alteration mediates many biological programs to provide an appropriate status for malignant cells' rapid proliferation (Garnis C, 2004). In this study, bioinformatics analysis by using high-throughput methods helps us provide useful information to predict how we can knock down the MSRB3 gene therapeutic target to increase apoptosis in cervical cancer.

Methods:

Microarray data analysis

The Human Protein Atlas (HPA) database (Uhlén, 2015) was used in the present study to check the unfavorable protein levels in cervical cancer tissues. To identify the differentially expressed genes (DEGs) in cervical cancer and the relationship between gene expression and cancer stage, TCGA data were used using the OncoDB database (Tang et al., 2022). Genes with adjusted<0.001 and |log2 FC|>1 are considered DEGs. Then the DEGs were shared in each group in three data series using the Venn diagram to identify the most predominant molecular alterations. The Kaplan-Meier survival plot in OncoDB clinical analysis validated the gene (MsrB3) survival rate with prognostic data.

Functional annotation analysis

The search tool for retrieval of interacting gene (STRING) database (Szklarczyk et al., 2019) was applied to predict functional interactions of proteins with MSRB3(methionine sulfoxide reductase B3). Active interaction sources, experiments, and databases, species limited to "Homo sapiens," and an interaction score>0.4 were applied to construct the PPI networks.

Gene ontology (GO) and pathway enrichment analysis were performed using Enrichr to explore the mechanism of MSRB3 in cervical cancer (Data extraction from Reactome 2016). Functional enrichment analysis was accomplished using the Gene Ontology analysis in the enrichr tool (Kuleshov et al., 2016) done for biological processes (BP), molecular function (MF), and cellular components (CC) categories. Terms with adjusted p-values less than 0.05 were reported as significant.

Prediction of a microRNA and transcription factor as a gene regulator

The TRRUST database established HSF2 as a transcription factor (TF)-target transcriptional network (Han et al., 2015). The online databases miRWalk were used for miR-331-3p, miR-338-3p, and hsa-let-7b-3p target prediction. Expression level analysis of miR-331-3p, miR-338-3p, and hsa-let-7b-3p in cancers was performed using the online database OncomiR (http://www.oncomir.org/)(Wong N, 2018) to gain an overall understanding of miR-331-3p, miR-338-3p, and hsa-let-7b-3p in cancer.

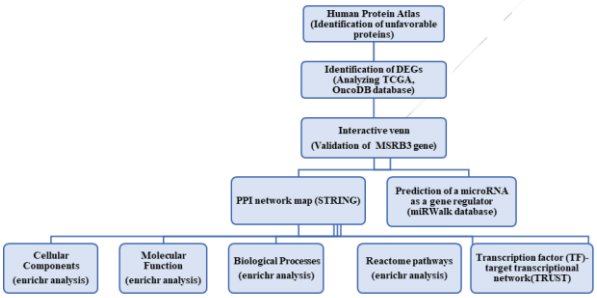


Fig. 1 - Research methodology flow chart

Results:

Identification of differentially expressed genes

A total of 1000 DEGs between normal and cancer samples was identified in the cervical squamous cell carcinoma adenocarcinoma expression datasets using OncoDB (p-value =< 0.001). The Unfavorable HPA group included 223 unfavorable proteins in cervical cancer. The clinical Stage group included 627 genes whose expression levels are significantly different between patients stratified by the selected clinical parameter, as determined by Analysis of variance (ANOVA) tests (p-value =< 0.001).

The data from the human protein atlas (223 genes associated with an unfavorable prognosis) and DEGs compared to Differential Genes in the clinical stage (p-value 0.0001), and MSRB3 was obtained through the Venn diagram (Fig. 2) as a target gene (Table 1).

The Kaplan Meier survival plot shows that high MSRB3 gene expression is associated with lower survival rates and poor prognosis in cervical cancer (Fig. 3). OncoDB gene expression analysis of MSRB3 gene has shown that MSRB3 gene expression is significantly decreased in CESC patients' tissues compared to normal tissues is significantly (Fig. 4).

Table 1 -MSRB3 information in HPA (Uhlén, 2015)

General Information	
Gene name	MSRB3
Description	Methionine sulfoxide reductase B3 (HGNC Symbol)
Gene description	Methionine sulfoxide reductase B3
Protein class	Disease related genes Enzymes Human disease related genes Potential drug targets
Predicted location	Intracellular
Number of transcripts	13

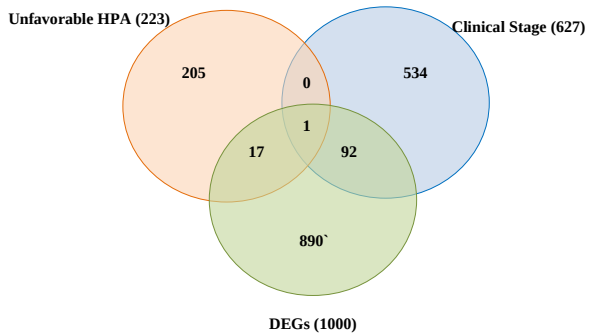


Fig. 2 - [Unfavorable HPA] and [Clinical Stage] and [DEGs]: MSRB3

Overlapping genes in human protein atlas (223 genes associated with an unfavorable prognosis) and DEGs (1000 genes) compared to Differential Genes in the clinical stage (627 genes) indicates MSRB3 as an upregulated differentially expressed gene (DEG) through the Venn diagram.

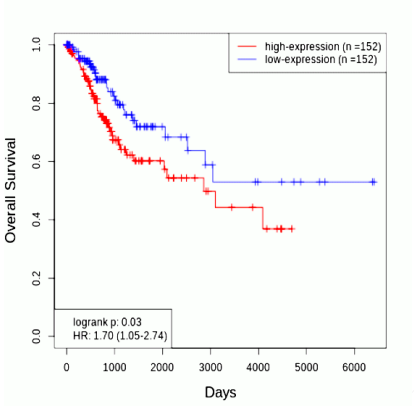


Fig. 3 - Survival plot

Gene: MSRB3

Cancer: CESC

Exp/meth: expression

Survival plot showing percent survival vs time are provided along with a MSRB3 gene expression and represented an association between overexpression of MSRB3 and lower survival rates and poor prognosis in cervical.

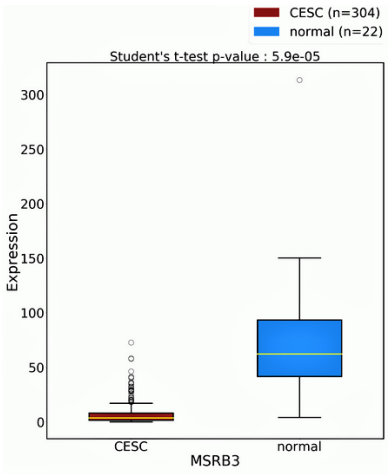
Fig. 4 - MSRB3 expression profile in OncoDB database

Cancer:CESC

Adjusted p-value:1.00e-03

|log2FC| (log2 transformed Fold Change):1

exp/meth:expression



Discovering associated gene ontology and pathways

To identify key proteins with regulatory impacts, the STRING was used for the PPI network construction. The PPI network map and Enrichr analysis (Reactome pathways, gene ontology) showed that the three genes (MSRB3, TXN, FXN) were closely related to each other and cancer pathways (Fig. 5). GO was analyzed by Enrichr in order to find out enriched cellular components, molecular function, biological processes, Reactome pathways, transcription factor (TF)-target transcriptional network (TRUST). Cellular_component Gene Ontology analysis demonstrated that FXN carries out a molecular function in the mitochondrial matrix and intracellular organelle lumen, and TXN carries out a molecular function in the nucleus and intracellular membrane-bounded organelle (table2). Further screening of GO molecular function revealed their role in oxidoreductase activity (Fig. 6). MSRB3, TXN, and FXN role in protein repair, and metabolisms of proteins were manifested which can have role in cancer progression (Fig. 7).

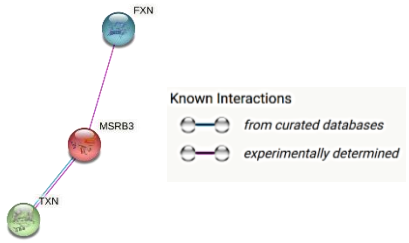


Fig. 5 - STRING: functional protein association networks

Active interaction sources: experiments, databases

Table 2- Cellular_component Gene Ontology

Term	Genes
mitochondrial matrix (GO:0005759)	FXN
intracellular organelle lumen (GO:0070013)	FXN
nucleus (GO:0005634)	TXN
intracellular membrane-bounded organelle (GO:0043231)	TXN
Term	Genes
mitochondrial matrix (GO:0005759)	FXN

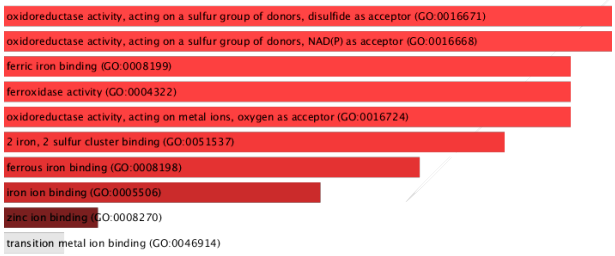


Fig. 6 - Gene ontology: GO Molecular Function 2021

sorted by p-value ranking.

Identification of MSRB3 gene regulators:

As mentioned in the previous paragraph, according to STRING, MSRB3 interacts with two additional genes (TXN and FXN). According to GO analysis, these genes were engaged in protein repair pathways and had oxidoreductase activity. Additional TRRUST analysis revealed that HSF2 is an upstream transcription factor (TF) that can be knocked down and regulate the expression of these proteins (Fig.7).

Also, using the miRWalk database, we predicted that miR-331-3p, miR-338-3p, and hsa-let-7b-3p microRNA regulate the MSRB3 gene (Sticht et al., 2018). Similar to previous experiments, miR-331-3p, miR-338-3p, and hsa-let-7b-3p expression is not uniform in different cancers and they may act as either an oncogene or tumor suppressor (Table 3), suggesting the functional diversity of miR-331-3p, miR-338-3p, and hsa-let-7b-3p in cancer development and malignant progression. There are 4 cancer types where survival is significantly associated with hsa-miR-331-3p which are adrenocortical carcinoma, cervical squamous cell carcinoma and endocervical adenocarcinoma, brain lower grade glioma, liver hepatocellular carcinoma. Notably, miR-331-3p is upregulated in living patients' cervical cancer tissues compared with deceased patients. miR-331-3p, miR-338-3p, and hsa-let-7b-3p overexpression and HSF2 as an upstream TF suppression has the potential to suppress the MSRB3 gene expression and by this way increase ROS-induced apoptosis due to the MSRB3 suppression.

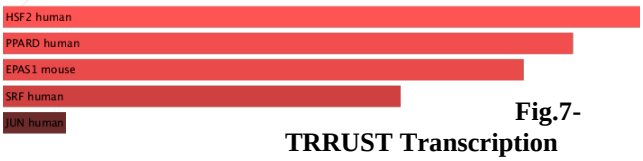


Fig.7- TRRUST Transcription Factors 2019

sorted by p-value ranking.

Table 3- hsa-miR-331-3p, hsa-miR-338-3p, hsa-let-7b-3p expression level in OncomiR

Cancer Abbreviation	miRNA name	Log2(Mean Expression)	Sample Number	Cancer Name
CESC	hsa-miR-331-3p	4.47	289	cervical squamous cell carcinoma and endocervical adenocarcinoma
CESC	hsa-miR-338-3p	8.93	289	cervical squamous cell carcinoma and endocervical adenocarcinoma
CESC	hsa-let-7b-3p	5.07	289	cervical squamous cell carcinoma and endocervical adenocarcinoma

Discussion:

Reactive oxygen species (ROS) play an essential role in apoptosis, a programmed cell death, by regulating the intrinsic mitochondrial apoptosis pathway (Fig.8) (Redza-Dutordoir & Averill-Bates, 2016). Apoptosis regulation is crucial for maintaining normal cellular homeostasis (Redza-Dutordoir & Averill-Bates, 2016). ROS can oxidize the sulfur-containing amino acid methionine, generating a diastereomeric mixture of methionine- (R, S)-sulfoxide that causes protein damage. Most living organisms have a repair mechanism involving the action of the methionine sulfoxide reductase (MSR) system (Ding et al., 2007). MSR is responsible for reducing methionine sulfoxide to methionine (Cao et al., 2018) (Fig.9). Because of the Msr system Met residues in proteins can also act as catalytic antioxidants by removing ROS. MSRB1 is a selenoprotein that can be detected in the nucleus and cytoplasm. MSRB2 and MSRB3, are translocated into the mitochondria and MSRB3 can also be found in the endoplasmic reticulum (Jiang & Moskovitz, 2018). In particular, overexpression of MSRB3 in fruit flies and mammalian cells increased resistance to oxidative stress. According to previous studies, depletion of MSRB3 causes apoptotic cell death in cancer cells (Kwak et al., 2017). Increased ROS and redox imbalance lead to MSRB3 down-regulation-induced cell death by activating apoptosis (Kwak et al., 2017). In this study, bioinformatics analysis confirmed Kwak et al. research and MSRB3 has been predicted as a protooncogene in cervical cancer by bioinformatics data analysis which can be regulated by a transcription factor or miRNA.

miR-331-3p is a member of the miR-331 family, located on 12q22n(Wang L, 2009). miR-331-3p has been shown to be involved in different cancers such as PCa, cervical cancer, glioblastoma, CRC, and HCC (Ng et al., 2019). mir-331-3p is necroptosis-related miRNA in cervical cancer and can accelerate the death of cervical cancer cells (Fujii et al., 2016). And also, numerous studies showed miR-338-3p as a putative tumor suppressor miRNA in different types of cancers, such as glioblastoma, renal carcinoma, gastric cancer, varian epithelial carcinoma, hepatocellular carcinoma (Hua, 2017b; Lu M, 2019; Wang et al., 2022). miR-338-3p is located on chromosome 17q25(Lu M, 2019). Although the potential benefits of miR-338-3p and miRNA-331-3p are proposed, the mechanism by which they can suppress cervical cancer is still unclear. Let-7 is the first miRNA studied in human malignancies. The Let-7 family has several members that act as tumor suppressors by down-regulating their target genes involved in multiple signaling cascades (Yang et al., 2008). Numerous studies have shown that low let-7 family miRNA levels in cervical cancer act as tumor suppressors by regulating several target genes (Liu & Ni, 2018). hsa-let-7b-3p is located on chromosome 22q13.31(Benson, 2013). In this study, bioinformatics analysis found that miR-338-3p, miRNA-331, and hsa-let-7b-3p can regulate the 3'UTR region of MSRB3 gene (Sticht et al., 2018) by reducing the expression of this gene, and show anti-cancer function. In conclusion, our results indicate miR-331-3p, miR-338-3p, and hsa-let-7b-3p overexpression can decrease the expression of MSRB3 as an unfavorable gene in cervical cancer.

HSF2is involved in regulating heat shock gene expression in cells undergoing differentiation and development processes. The heat shock response enables the cell to cope with the deleterious effects of protein-damaging stresses. The characteristic of the heat shock response is a general down-regulation of gene transcription and protein synthesis (Huang et al., 2018). In contrast, the transcription of a specific subset of genes called the heat shock genes is induced. Activation of HSF2 is required for induction of TNX, FXN, and MSRB3 gene expression. The oxidized forms of the MSR enzymes are reduced by the reduced form of TNX. Using these bioinformatics tools, we predicted the role of HSF2 transcription factor as a target to be knocked down to control TNX expression and MSRB3 function (Kuleshov et al., 2016).

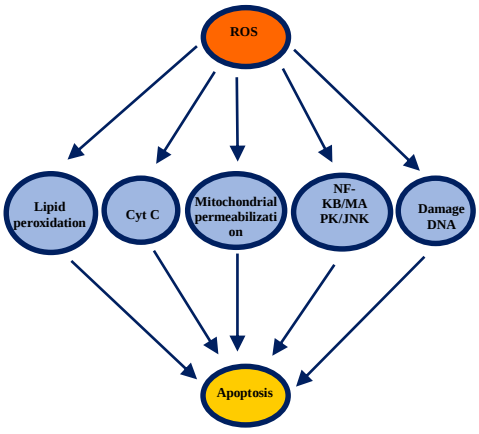


Fig.8 - Mechanisms underlying that ROS induced apoptosis (Zhao et al., 2019). Excessive ROS can cause apoptosis via multiple ways: ① enhancing lipid peroxidation. ② Releasing of Cytc. ③ Enhancing mitochondrial permeabilization. ④ Activation of NF-KB/MAPK/JNK pathway. ⑤ Damaging DNA directly

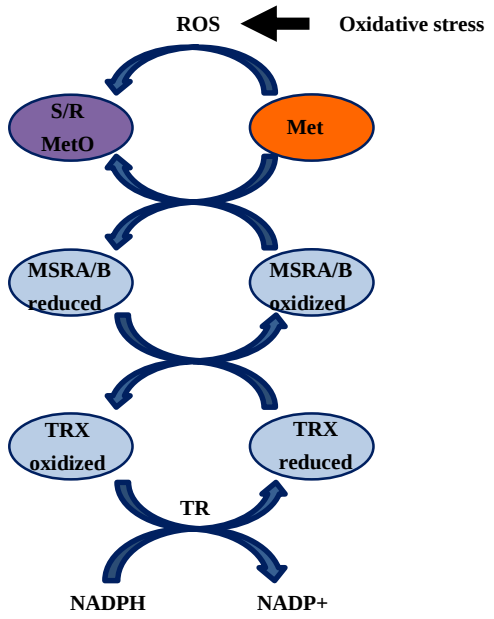


Fig. 9 - Schematic illustrating of the redox cycle of MSRA/B in reducing MetO. Oxidative stress causes the production of reactive oxygen species (ROS) that in-turn oxidize Met to S-MetO and R-MetO epimers. The reduced form of either MSRA or MSRB reduces S- or R-MetO respectively. The oxidized forms of the MSR enzymes are reduced by the reduced form of thioredoxin (TRX). The oxidized form of TRX is reduced by the enzymatic activity of thioredoxin reductase (TR) that uses NADPH for its function, resulting in the NADP+ production (Jiang & Moskovitz, 2018).

Conclusion:

We suggested that MSRB3 expression is associated with a poor prognosis and survival in cervical cancer patients by using microarrays with bioinformatics analysis. Thus, treatment strategies that target MSRB3 expression may prone cervical cancer cells to apoptosis. We predicted that the miR-331-3p, miR-338-3p, and hsa-let-7b-3p might be involved in regulating MSRB3 and other related proteins. In addition, using bioinformatics tools, we predict the role of HSF2 transcription factor as a target to control TNX expression and MSRB3 function in the course of tumor development in experimental design, in vitro and in vivo, to employ them for therapeutic approaches.

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Conflicts of interest

The authors declare no conflicts of interest.

Ethics approval

Not applicable

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