

Original Research**TARGETON TUMOR SUPPRESSOR GENES BY miR-141 FAMILY AS A POTENTIAL REGULATORY FUNCTION IN CERVICAL CANCER**Emad Dabous¹, Adel A. Guirgis¹, and Hany Khalil^{1*}

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Abstract. Introduction: Historically, microRNA is a nanosecond order of single beachfront RNA, hairpin in structure, and roughly 22 nucleotides in the mature phase. Before three decades ago, we did not know about this crucial cellular element. The primary function of miRNAs is targeting and regulating certain gene expressions; therefore, the miRNAs are involved in various human diseases and medical disorders, including infectious diseases and cancer. Identifying potential targeted genes by specific miRNA is essential to recognize, investigate, and treat any related illness. Notably, the miR-200 family, including a cluster of miR-200a, miR-200b, miR-429, and miR-141, has been reported as an essential miRNA aberrantly expressed in several human cancers and involved in cancer initiation and invasion, angiogenesis and metastasis, and cancer diagnosis and therapy.

Methods: PicTar (<https://pictar.mdc-berlin.de/cgi-bin/PicTar Vertebrate.cgi>), an algorithm, was used to identify microRNA targets.

Results: In this bioinformatics-based study, we identified the potential targeted genes by the miR-200 family using PicTar software to predict the molecular function of the miR-200 family in cancer development. The most identified targets with low required energy and high PicTar score include a variety of tumor and metastasis suppressor genes, such as the metastasis suppressor gene MTSS1 deleted liver cancer -1 (DLC-1) that are regulated by miR-200a and miR141, respectively. The deficient energy required for targeting these genes and completing the interfering reaction makes it a straightforward and spontaneous cellular event.

Conclusion: The miR-200 family by its members, especially mi, strongly impacted cancer development and metastasis types, including cancer cervix.

Keywords: : microRNA, Gene regulation, Cancer, Prediction tools

INTRODUCTION. Noteworthy, microRNAs (miRNAs) are small noncoding RNA, approximately 18-23 nucleotides that can post-transcriptionally regulate the expression of its cleaved messenger RNAs (mRNAs). Hundreds of miRNA genes have been identified in diverse animals and many phylogenetically conserved species [1]. In mammalian cells, miRNAs are up-regulated upon several microbial infections to modulate a variety of intracellular signaling [2]. Other cumulative evidence indicated that some

endogenous miRNAs are able to interact as a tumor suppressor or oncogenes during cancer development [3]. Like all nucleic acid products, miRNA is transcribed by RNA polymerases following the life law (DNA) as the genome contains every miRNA and its specific sequence regulation. It starts with a recap of the DNA rendering region of microRNA by RNA polymerase II, also an extension of the transcriptome, the hairpin formed and the pre miRNA circumscribing at 5- end, and polyadenylation of 3- end to start the third necessary process in which splitting of hairpin and RISC complex conformation is performed [4]. Mammalian RNA polymerase III paraphrases some types of noncoding RNA, such as Alu sequences [5]. DNA is not the final template of miRNA; about 640 mortal miRNAs are under editing or called iso-miRNAs; this fact was chased by Morin and his collage in 2008 [6].

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Launching tumorigenic processes mediates the neoplasia of normal cells, potentially performing in the inauguration of malice. Strict mechanisms applicable to the incident or series of different cancers can be subordinated to clinical operation, such as early forestallment, webbing, or specific treatment [7]. Between all nonsupervisory factors involved in altered gene expression linked with carcinogenesis, the impudence of miRNAs has been astronomically pursued for decades [8]. MiRNA is a noncoding RNA almost 22nt in length that functions as a post-transcriptional regulator for forfeiture tuning the rendering effectiveness of runner RNA (mRNA). Targeting of the RNA-convinced silencing complex (RISC) formed from single-beachfront miRNA, Argonaut (AGO), and GW182 (also known as TNRC6A) mediates the translational suppression or declination of the nonsupervisory seeker. miRNA-intervention regulation applies to eclectic cellular or environmental stress, including starvation, oxidative stress, hypoxia, and DNA breakdown, thereby being concerned with nasty complaints [9].

Consequently, the deregulation of miRNA expression is honored to parade a bidirectional effect toward oncogenesis or excrescence repression [10]. Disturbance in miRNA expression can be caused via multiple ways, including transcriptional regulation, epigenetic methylation of miRNA-containing loci, miRNA processing pathway, and insulation with long noncoding RNA, which functions as the miRNA mop [11]. For illustration, various p53-responsive miRNA networks containing miR-34 or miR-27b are proven to intervene in the quiescence of distinctive cancer cells. Mutant p53 reversely reduces the excrescence suppressive impudence of p53-regulated miRNAs on carcinogenic hand [12]. Recent studies expose the correspondence between miRNA expression and epigenetic control regarding CpG island methylation within the protagonist region in cancer. The silence of miR-127, miR-124-1, or miR-129-2 is nearly related to the hypermethylation of CpG islet-containing protagonists in colorful solid cancers [13]. In addition, the function or expression of miRNA processing ministry, similar to Drosha or the DGCR8 protein, is constantly deregulated in different malice. Indeed, though the impact of Drosha or DGCR8 on carcinogenesis is controversial, the disturbance of the miRNA processing ministry is nearly related to the global change in the miRNA expression biographies [14]. Cancer-intermediated genomic insecurity results in modifying miRNA loci, leading to the miRNA dupe number variation. Whereas the significance of miRNAs in mortal carcinogenesis is decreasingly honored, the understanding of the implicit part of miRNAs in cervical carcinogens is still

limited but supposed by several studies [15]. Likewise, a study reported that high-threat mortal papillomaviruses (HR-HPVs) are responsible for the upregulation of oncogenic or downregulation of excrescence suppressive miRNAs, and these compliances could exfoliate further light on HR-HPV- convinced oncogenesis. In addition, it has shown a discriminational expression of mature miRNAs during the successive stages of cervical scaled cell melanoma (SCC) development; these compliances suggest that numerous aberrantly expressed miRNAs in cervical cancer could serve as individual and prognostic biomarkers [16]. Based on this, this study aims to provide prediction tools that facilitate the identification of potentially targeted genes by the miR-200 family and highlight the possible regulatory role of this family of miRNA in regulating cancer.

METHODS. PicTar ([https://pictar.mdc-berlin.de/cgi-bin/PicTar vertebrate.cgi](https://pictar.mdc-berlin.de/cgi-bin/PicTar Vertebrate.cgi)) is an algorithm for the identification of microRNA targets. This searchable website provides details (3'UTR alignments with prognosticated spots, links to colorful public databases) regarding miRNA target prognostications in invertebrates, microRNA target prognostications in seven *Drosophila* species, microRNA targets in three nematode species, and mortal miRNA targets that are not conserved but-expressed this means that the miRNA and mRNA are expressed in the same towel [17].

RESULTS. Potentially targeted genes by miR-141 are implicated in cancer development and metastasis. By using optimal tools in miRNA prognostications, as shown in Figure 1, we plant that miR-141 targets numerous genes, such as the DCL1 gene, which is responsible for cancer liver development, in two suggested seeding regions (SR); the first one started at the passion 1700nt (CAGTGTT) with demanded energy (-20.7), the alternate point created at the passion 1710nt (CAGTGTT) with response energy-15.9. The FUS gene impacted liposarcoma in the suggested targeted end CAGTGTT with the energy needed -18.9. PDCD4 gene, as one of the neoplastic impediments, is potentially targeted by miR-141 in a point at 506nt (CAGTGTT) with required energy -24.1. NSE2 gene controls the cancer membrane protein and is targeted by miR-141 in presented fact CAGTGTT (3021nt) with energy -21.9. DCL1S2 gene, as one of the diurnals of esophageal and lung cancer suppressor gene, is targeted by miR-141 at seeding region ACAGTGT with required energy -14.3. Likewise, the metastasis suppressor gene MTSS1 is suggested to be targeted by miR-141 at 811nt (CAGTGTT) with energy -20.4. The data shows that the sowing region with eight nucleotides in

about all targeted genes and the deficient energy needed in the targeting process make this natural process an accessible robotic cellular event (Table 1).

needs -18.8. The metastasis suppressor gene MTSS1 gene is also potentially regulated by miR-200a by a seeding site indicated at 811nt (CAGTGTT), needing energy -20.7.

	Target gene	Seeding region	PicTar score	Disease related	Free energy
miR 141	DCL 1	CAGTGTT (1700) CAGTGTT (1710)	6.67	Liver cancer	-20.7 -15.9
miR 141	FUS	CAGTGTT (46)	4.07	Liposarcoma	-18.9
miR 141	PDCD4	CAGTGTT (506)	3.68	Neoplastic inhibitor	-24.1
miR 141	NSE 2	CAGTGTT (3021)	3.68	Brest cancer membrane protein	-21.9
miR 141	DLEC1-S2	ACAGTGT (10)	2.66	Lung and esophageal cancer	-14.3
miR 141	MTSS1	CAGTGTT (811)	2.63	Metastasis suppressor	-20.4

Table 1: Potential targeted genes and seeding regions for Has-miR-141 indicated by PicTar software.

These genes predispose directly to neoplasm diseases such as liver cancer, liposarcoma, or indirectly as a neoplastic inhibitor or metastatic suppressor. The deficient energy required for targeting reaction makes it a straightforward and spontaneous cellular event. There are also SRs of about seven nucleotides in some genes; more than one seeding region increases the targeting liability.

Targeted genes by miR-200a regulates programmed cell death and metastasis in cancer cells. As shown in Figure 2: miR -200a targets many genes to control oncogenetic cellular activity, for example, the DCL1 gene, which is responsible for hepatocellular carcinoma development by two suspected seeding region (SR1) AGTGTTA, at 1701nt, with energy -24.8, and another SR at CAGTGTT (1790NT) with energy needed -15.3. PDCD4 gene regulates programmed cell death and initiation of oncogenetic activity, also targeted by miR-200a at position 506nt (CAGTGTT) with energy -21.5. PLAG1 gene, which impacts adenomas growth, is targeted by three seeding regions AGTGTTA (3177nt), CAGTGTT (4542nt), AGTGTTA (6311nt), with energy -22.7,-17.5,-23.0 respectively. Likewise, miR-200a targets the NSE2 gene in breast cancer membrane protein CAGTGTT 3021, with energy-free

MiR200c also targets some genes as his family members miR200a and miR141, by two famous examples DOC1 gene one of genetic series of ovarian cancer with seeding site CAGTATT and energy -22.0 and MTUS1 gene at indicated seeding region CAGTATT 1727 and energy free -17.4 (Table 2). Together, genes targeted by miR-200a include DCL1, PDCD4, PLAG1, NSE2, RAB11, and MTSS1. These may cause or strongly impact hepatocellular carcinoma, neoplasm transformation, adenoma, breast cancer, lung cancer, and metastasis in the case of MTSS1 gene targeting. The low energy consumed in targeting reaction and more than one seeding region as PLAG1 give more support for the reality and chances of this cellular event to proceed.

The potential seeding regions and binding affinity of hsa-miR-200a on targeted genes were carried out by PicTar software: (A) Two detected seeding regions on STAT3 sequences (SR1 and SR2). (B) Two detected SR on PPARA gene sequences. (C) The indicated binding site of miR-21 on TGF- β . (D) TNF-SF6 binding affinity with miR-21. (E) CDC25A-miR-21 potential binding site. (F) PDCD4-miR-21 indicated seeding regions.

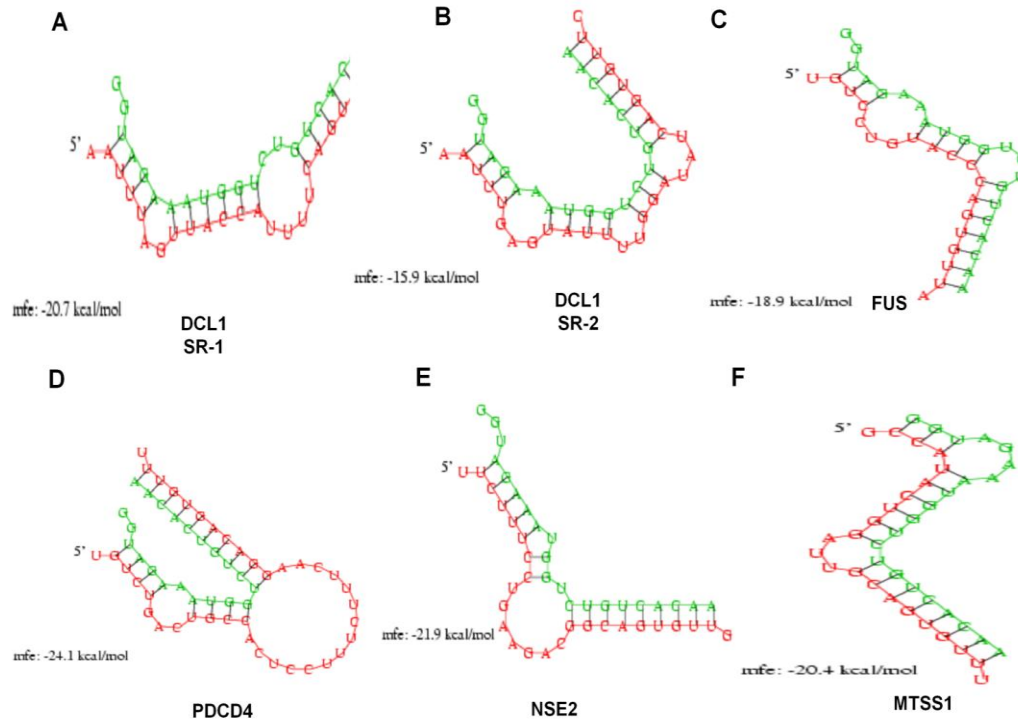


Figure 1. Predicted genes that potentially targeted by hsa-miR-141.

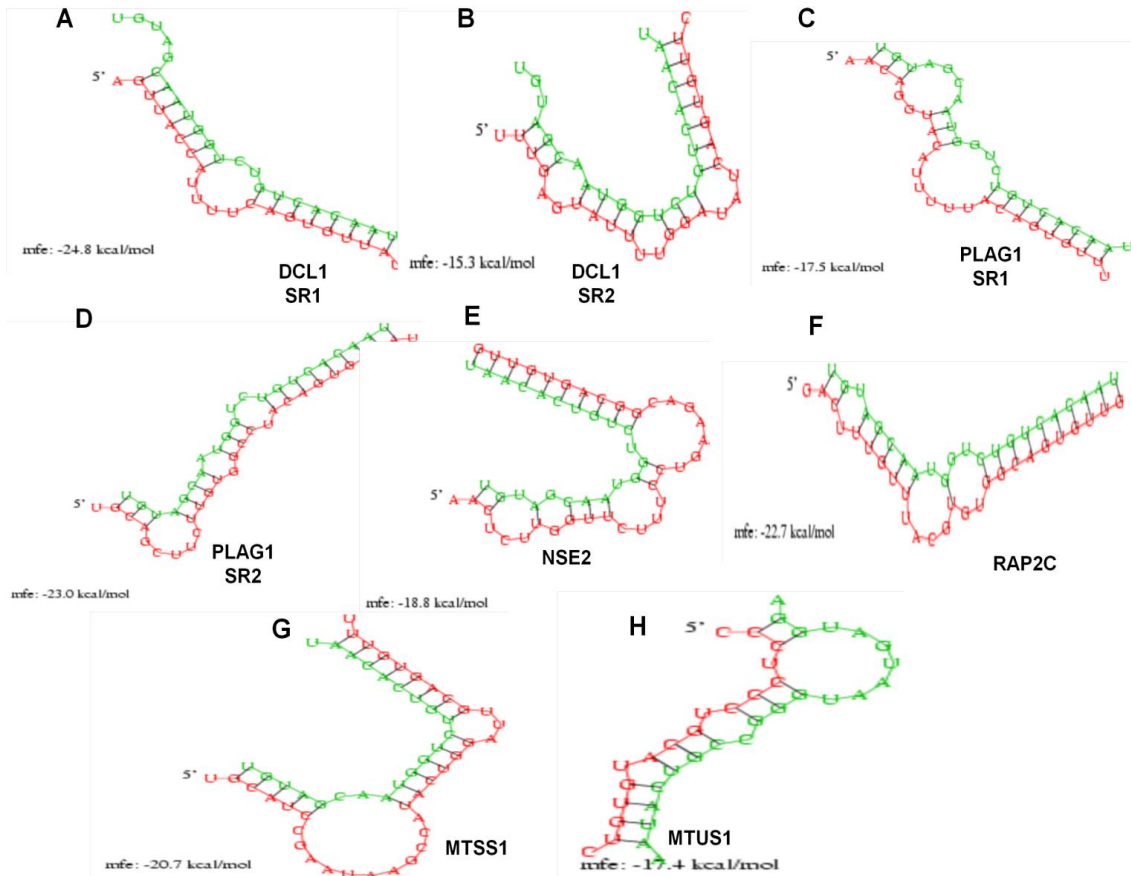


Figure 2. Predicted genes that potentially targeted by hsa-miR-200a.

miR	Targeted genes	Seeding region	PicTar score	Related disease	Free energy
MiR 200a	DCL1	AGTGTTA 1701 CAGTGTT 1790	6.39	Hepatocellular carcinoma	-24.8 -15.3
MiR 200a	PDCD4	CAGTGTT 506	3.68	Neoplasm transforming initiation in all tissues	-21.5
MiR 200a	PLAG 1	AGTGTTA 3177 CAGTGTT 4542 AGTGTTA 6311	3.68	Adenoma gene	-22.7 - 17.5 - 23.0
MiR 200a	NSE2	CAGTGTT 3021	3.55	Brest cancer membrane protein	-18.8
MiR 200a	RAP2C	CAGTGTT 556 CAGTGTT 2321	3.01	RAS oncogenes family, lung cancer	-17.2
MiR 200a	MTSS1	CAGTGTT 811	2.63	Metastasis suppressor gene	-20.7
MiR 200c	DOC1	CAGTATT 298	2.79	Ovarian cancer	-22.0
MiR 200c	MTUS 1	CAGTAT 1727	2.03	Clear cell carcinoma, Hepatocellular carcinoma.	-17.4

Table 2. Potential targeted genes and seeding regions for Has-miR-200a indicated by PicTar software.

DISCUSSION. In malignancy of inclusive textbooks that described the molecular function of the miR-200 family, the exact part of these miRNAs in cancer is still partly understood, with numerous studies suggesting further predominant onco-suppressive mechanisms. In contrast, other reports suggest possible oncogenic functions [10]. For illustration, drop miR-200b/ c expression in cancer bone was identified with a drop survival period, whereas increased miR-200a expression was identified with distant metastases. miRNA can participate in several steps of the metastatic process. It is possible to make an outcome prognosis by comparing miRNA expression in normal bone towels, in situ melanoma, non-metastatic, and metastatic bone cancers. It also established that the miR-200a/b family was increased in metastatic excrescences compared to non-metastatic cancer [7].

It is well known that miRNAs can be upregulated or downregulated in colorful mortal cancers [18]. Overexpressed miRNAs in cancer may serve as oncogenes and promote cancer development by negatively regulating excrescence suppressor genes and/ or genes that appreciatively control cell isolation or apoptosis. In contrast, under-expressed miRNAs in cancer function as excrescence suppressor genes and may inhibit cancers by

regulating oncogenes and/ or genes that control cell isolation or apoptosis [19]. Upregulation of miRNAs in mortal cancers can be affected by modification, deregulation of a recap factor, or demethylation of CpG islets in the protagonist regions of the corresponding genes. MiRNAs acting as excrescence suppressors can be downregulated in cancer by elisions, epigenetic silencing, or loss of recap factor expression [20]. In multitudinous cancers, including cancer cervix, miR-141 is over-expressed. Lately, it has been shown that miR-141 downregulated the expression of TGF2, which shows reduced expression in Cervical Cancer [11]. This suggests that the overexpression of miR-141 in the cervical epithelium could thus play a part in cervical excrescence neoplasm [21].

Metastasis is still a vast defy in cancer operations. The pathways essential in cervical cancer metastasis are not well understood till the jotting of these words. Numerous studies stressed the varied places of miRNAs in cancer development and metastasis. Adding figures of miRNAs, lncRNAs, and circulating miRNAs are plants to be deregulated in cancer cervix, confederated with metastasis. It regulates metastasis by controlling metastasis-related genes, epithelial-mesenchymal

transition, signaling pathways, and relations with the excrescence micro-environment [15]. It was discovered that miR-200a greases the metastasis of ovarian and cervical cancer telomere-associated poly (ADP-ribose) polymerase (PARP) family, which contributed to lengthening telomeres length and enhancing lump growth [15]. MiR-200a is known to be directly over-regulate TNKS2 and eventually supports the metastasis and irruption of cervical cancer [22]. Likewise, programmed cell death protein 4 (PDCD4), inhabited by miR-141, was attributed to the repression of cancer cell metastasis and irruption. miR-200b and miR-200c, along with miR-429, were linked as upstream of the AT-rich interactive sphere-containing protein 1A (ARID1A), which is concerned in SWI/SNF family and honored as an excrescence suppress or in cancer in the course of numerous mechanisms and pathways, similar as p53 and KRAS pathways [23]. Also, the auspicious part of miR-200c and miR429 positively affects the metastasis mechanisms of the cervical. Also, miR-10a and miR-590 were identified with the expression of a member of cell adhesion motes (CAMs), especially CCAM3, CCAM6, and CCAM7, which results in increased metastasis and irruption of cancer cells. The migrant and invasive capabilities of cancer cells could also be actuated by miR-501 by lowering the expression of cycling dermatosis (CYLD) and stimulating NF activation [24]. MiR-92a functions as an onco-miRNA by targeting the F-box and WD reprise sphere-containing 7 (FB XW 7), thereby elevating the metastasis of cervical cancer [25]. The upregulation of miR-181a was linked with the progression of cervical cancer through unfavorably targeting inositol poly phosphate-5-phosphate A (INPP5A) [26]. MiR-19a and b were well-known to be expressed further than normal in cancer cervix and promote cell irruption through both direct and negative control of Cullin-5 (CUL5) expression, which is known as vasopressin- actuated calcium marshaling receptor (VACM-1) [12]. Numerous studies picked up the part of miRNAs in tumorigenesis miracle; in the current study, we try to give an important participation to the former studies. According to use tools (web spots), we proved that miRNA 200 family targeting cancer-related genes like DLC1, FUS, NSE2, PDCD4, DLC1S2, and MTSS1. All these genes are involved in one or more types of cancer [27, 28].

CONCLUSION. Our study focused on genes targeted by the miRNA 200 family. Many genes contribute as a target of the miR-200 family; we only select a sample containing genes responsible for neoplasm development and progression. By the way, we try to find the prediction sites in these genes to enhance the knowledge about the role of the miR-200 family in neoplasm formation. The figures

and tables summarize multiple examples of the miR-200 family and their targeted genes according to software prediction tools on specific websites. All models selected by aiming at cancer relate to a selected gene or at least the role of the gene in tumor development and growth. We can conclude that the miR-200 family by its members, especially miR-141, strongly impacted cancer development and metastasis types, including cancer cervix.

Conflicts of interest: Authors declare no conflict of interest.

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Authors' contributions: Emad Dabous performed the analysis. Adel Guirgis helped in supervision. Hany Khalil designed the research plan, interpreted, and organized the results. Emad Dabous wrote the manuscript.

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