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Research Article

Expression Profile of Cancer-Related miRNAs in HeLa Cervix Carcinoma Cells

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Abstract

Background: Cervical cancer (CC) is the second leading common cancer among women globally. The disease begins with abnormal changes in the cervical that is generally associated with infection with human papillomavirus (HPV). Studies indicated the crucial role of miRNAs in CC tumorigenesis, progression and metastasis. More than 40 miRNAs have been reported signifying their role in the regulation of CC.

Material and Methods: In the present study, the expression profiles of 24 miRNAs were measured using PCR array for tumor suppressor genes.

Results: We have reported 9 upregulated miRNA (hsa-miR-31-5p, hsa-miR-23b-3p, hsa-miR-30d-5p, hsa-miR-206, hsa-miR-20b-5p, hsa-miR-30c-4p, and hsa-miR-145-5p) and 15 downregulated. Using miRNet online prediction tool (link provided in the M and M section), genes, diseases, lncRNA, and small molecules were predicted for the upregulated group only. Data obtained indicated that 8 of the upregulated miRNAs in HeLa cervical cancer cells targets 2429 genes, two of them targets 20 different diseases, three of them target 31 small molecules, and eight of them targets 253 lncRNAs.

Conclusion: The present study revealed that hsa-miR-20b-5p could be used as a potential biomarker because of its high expression profile in CC cells.

Keywords: miRNA; Cervical Cancer; Hela; Profiling

Introduction

Cancer is health-threatening large group of diseases that is considered the second leading cause of death worldwide, with nearly one in each six individuals dies due cancer [1-3]. This disease is practically the main cause of about 9.6 million deaths in the year 2018. About 70% of deaths due to cancer occurs in low- and middle-income countries [4].

Cervical cancer (CC) is a disease that affects females, where it is diagnosed from the age 35 to 45 [5]. Less than 20% of all cases were diagnosed in females over the age 65 [6,7]. Deaths due to CC were estimated to be 0.7% of all cancer-related deaths [8]. Several etiologies predispose to cervical cancer, including human papillomavirus (HPV) infection, smoking, obesity, prolonged use of oral contraceptives, intrauterine device use, economic status, multiple pregnancies, and family history of the disease [9,10].

Several studies highlighted the early diagnosis of CC using various techniques, which includes colposcopy, cone biopsy, computed tomography (CT), magnetic resonance imaging (MRI), intravenous urography, and positron emission tomography (PET scan) [11-14]. Nevertheless, epigenetics-based diagnosis approached might also be used recently due to its precision and straightforwardness [15]. One of the molecular tools used to early diagnose CC is miRNA profiling. Various studies identified changes in specific miRNAs expression profile in serum, along with the characterization of the methylation landscape of these miRNAs a number of unique miRNA that were shown to be dysregulated in patients with CC [16-19].

Furthermore, searching for specific miRNAs that could be used as a potential predicting biomarker in CC is demanding. Several research groups have attempted to identify miRNA panel they might underlie or predispose to CC. These studies found three miRNAs with prognostic value: miRNA-218-1, miRNA-145 and miRNA-200c [20]. Other studies indicated several upregulated miRNAs in CC biopsies compared with normal tissues, including miR-196a, miR-27a, miR-21, miR-34a and miR-22 [21,22]. Meanwhile, miR-126, and miR-143 were also highlighted for their diagnostic value [23,24]. The progression of CC was also found to be associated with specific miRNAs such as miR-34a, miR-125 and miR-375, and miR-19a and miR-19b [25].

Aim of the Study

In this study, we aimed to indicate the up- and down-regulated miRNAs in HeLa cervical cancer cells. This might help in reaching a reliable, validated miRNA for early warning of CC.

Materials and Methods

Cell line culture and maintenance

Cervix carcinoma cell line (HeLa) was purchased from the Holding Company for Biological Products and Vaccines (VACSERA), Giza, Egypt. Cells were maintained under the normal laboratory conditions i.e. 37 °C and 5% CO_2. Cells were grown in DMEM supplemented with 10% FBS and 1% antibiotic mix.

Harvesting cells

Attached cells were trypsin zed using 0.05% Trypsin EDTA (0.53 mM) and centrifuged for 10 minutes at 13,000 rpm at 4°C. Detached cells were washed in PBS and 1% BSA twice. The cell count was normalized (diluted/concentrated) to 1 x 10^6 cells mL^{-1}.

miRNA extraction and cDNA synthesis

Total micro-RNA was extracted was extracted using miRNeasy Mini Kit (QIAGEN, Germany). The extracted miRNA was then converted to cDNA using miScript II RT Kit (QIAGEN, Germany).

PCR array panel

In the present study, miScript miRNA PCR Array Human Tumor Suppressor miRNAs (GIAGEN, Germany) containing 24 different miRNA along with U6 snRNA as housekeeping genes was used.

Real time amplification

Real time PCR (Step One Plus, ABI) was used to amplify 24 miRNAs in the HeLa cells. Two micrograms of cDNA were loaded to each well in the 24-well real time PCR plate. Master mix was added following the manufacturer protocol. The thermal profile involved pre-heating at 95°C for 5 minutes and the cycle program was 94°C for 30 seconds, 58°C for 45 seconds and 72°C for 45s a final extension step was involved at 72°C for 5 minutes.

Target prediction

The upregulated miRNAs were uploaded to miRNet online web generator tool (http://www.mirnet.ca/faces/home.xhtml) to identify the target gene, disease, lncRNA, and small molecules.

Statistical analysis

Statistical analysis was performed using SPSS software package (SPSS, Inc., Chicago, IL). Obtained values were expressed as mean ± SD. Analysis of variance with t test was used to calculate the significance of the difference. P value equals or of less than 0.05 was considered statistically significant.

Results

Downregulated miRNA

In the present study, 24 different miRNAs were profiled in HeLa cervix cancer cells. Of this panel, 15 miRNAs were found to be downregulated compared to the HKG U6 snRNA (Figure 1). Only seven miRNAs were significantly (P = 0.05) differed from U6 snRNA. These miRNAs were hsa-miR-196a-5p, hsa-miR-21-5p, hsa-miR-141-3p, hsa-miR-99a-5p, hsa-miR-133a, hsa-miR-103a, hsa--miR-19a-3p, and hsa-miR-194-5p.

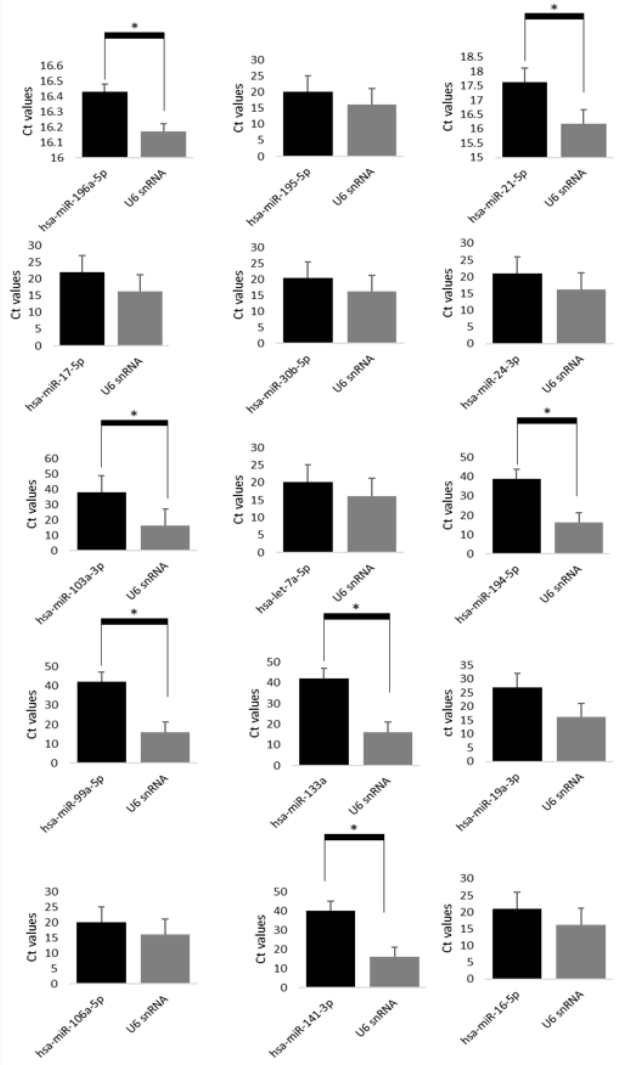

Figure 1: Downregulated miRNAs in HeLa cervix cancer cells compared to U6 snRNA.

Upregulated miRNA

In the cervical cancer cells, nine members of miRNA panel used were found to be upregulated, compared to U6 snRNA. Significantly, upregulated miRNAs were hsa-miR-31-5p, hsa-miR-23b-3p, hsa-miR-30d-5p, hsa-miR-206, hsa-miR-20b-5p, hsa-miR-30c-4p, and hsa-miR-145-5p (Figure 2).

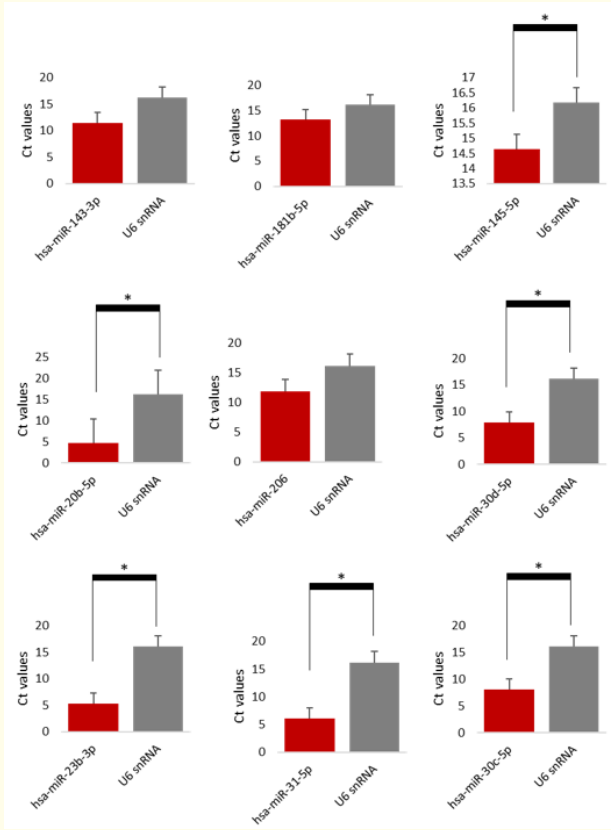

Figure 2: Upregulated miRNAs in HeLa cervix cancer cells compared to U6 snRNA.

Predicted gene interactions

Using the miRNet online tool (degree filter 1.0 and "All network nodes" option was selected, betweenness filter was 0.0 and "All network nodes" option was selected), 8 miRNAs (out of 9 upregulated miRNAs) were found to target 2429 genes in the human genome [26]. These miRNAs were hsa-miR-106a-5p, hsa-miR-145-5p, hsa-miR-17-5p, hsa-miR-21-5p, hsa-miR-23b-3p, hsa-miR-24-3p, hsa-miR-30d-5p, hsa-miR-99a-5p, hsa-miR-133a-3p, and hsa-miR-16-5p (Figure 3).

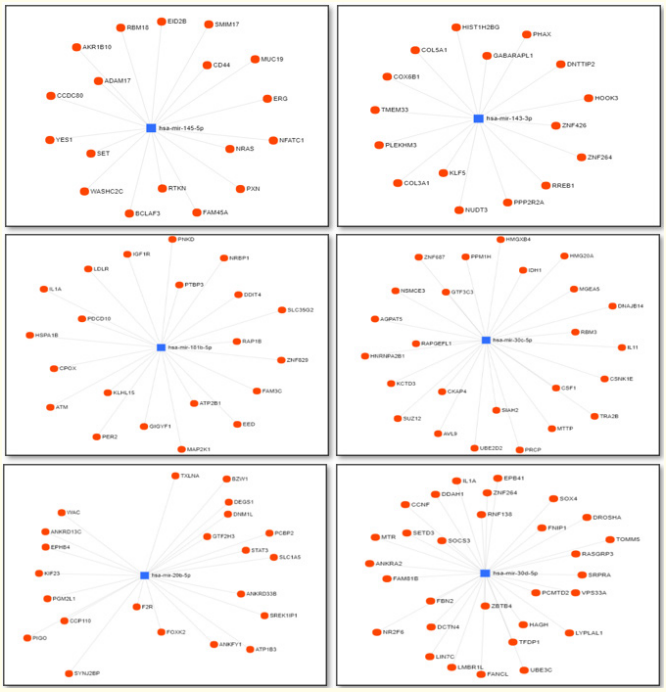

Figure 3: Nine-upregulated miRNAs that interact with several genes based on the prediction tool miRNet.

Predicted diseases interaction

Two upregulated miRNAs (hsa-miR-30c-5p and hsa-miR-181b-5p) in HeLa cells were found to target 20 different diseases such as breast cancer, glioblastoma, pancreatic cancer, colorectal cancer, bladder cancer and mouth cancer, among others (Figure 4).

Predicted small molecules

By profiling cervical cell line, only three miRNAs (hsa-miR-145-5p, and hsa-miR-23b-3p, and hsa-miR-30d-5p) among the upregulated groups were found to be interacting with 31 small molecules such as trastuzumab and cisplatin (hsa-miR-30d-5p), 5-aza-cytidine and 5-flurouracil (hsa-miR-23b-3p), and vemurafenib and temozolomide (hsa-miR-145-5p) (Figure 5).

Predicted lncRNA

Out of the upregulated miRNAs, eight were found to target 253 long non-coding RNAs, applying the same parameters on the miRNet tool (Figure 6).

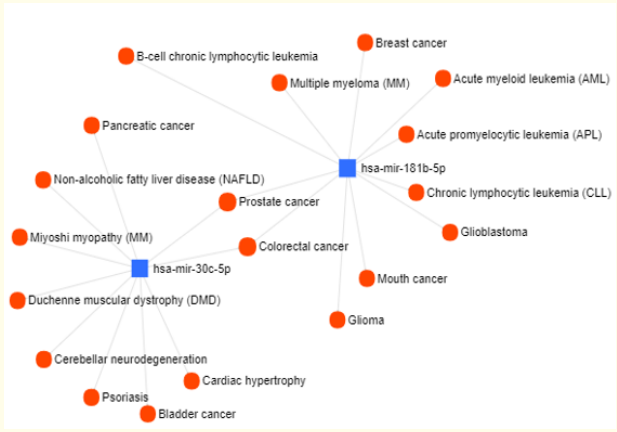

Figure 4: Two-upregulated miRNA in cervix cancer cells interacts with 20 different diseases including several types of cancers.

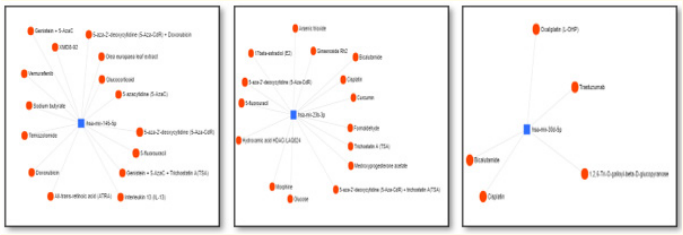

Figure 5: The three upregulated miRNAs predicted interaction of with small molecules.

Predicted epigenetic proteins

The upregulated miRNA showed no interaction with any epigenetic proteins according to the above-mentioned parameters and settings.

Discussion

Predicted genes

In the present study, a panel of 24 miRNAs was profiled in HeLa cervical cancer cells. The upregulated miRNAs were submitted to miRNet online web generator tool to predict the interaction with target genes. Data revealed that, out of these 24 miRNAs, only three were found to target 892 genes. This might indicate the significant role miRNA plays not only in the carcinogenesis process, but also in

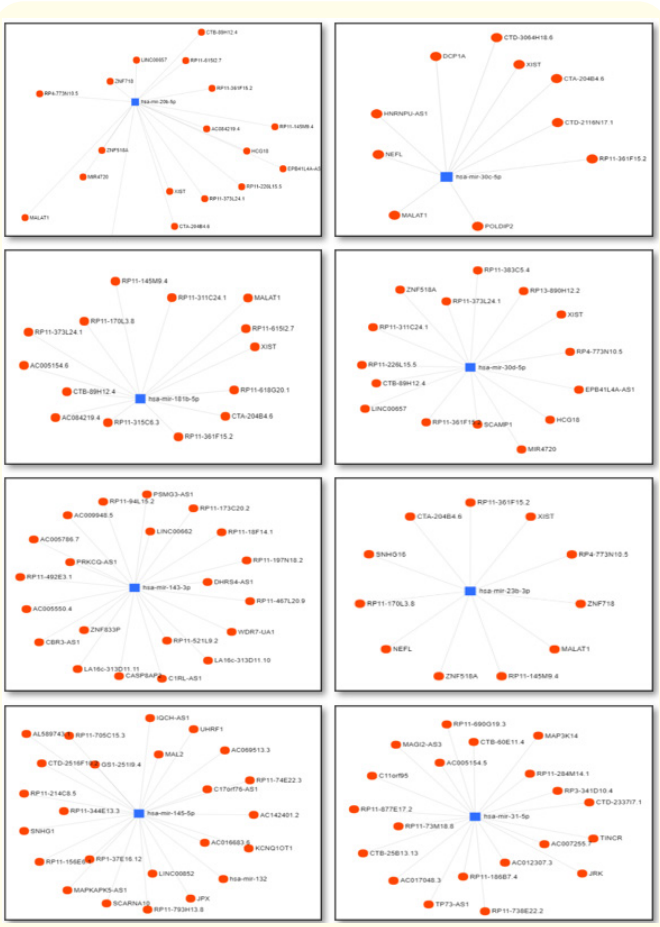

Figure 6: The eight upregulated miRNAs interact with 253 lncRNAs.

various cellular activities [27,28]. Target genes fall into several categories: TSG (*P53*, *Rb*, and *P21*), cell cycle regulator (*CDK* family), membrane proteins (*CDH1*), signal transduction proteins (*GSTP*), and apoptosis-related proteins (*BCL*-2, *BAX*).

Predicted diseases interaction

Here, hsa-miR-30c-5p and hsa-miR-181b-5p were found to be correlated with several diseases including cervical carcinoma. Several studies showed that hsa-miR-miR-30c-5p is highly expressed in breast cancer [29], colorectal carcinoma [30], hepatic carcinoma [31], cardiomyopathy, and ovarian cancer [32]. However, this miRNA is not only associated (upregulated) in these diseases, but rather it has a function in the development and progression of almost all types of cancer [30,33,34]. Therefore, it could not be employed as a biomarker for CC.

Meanwhile, hsa-miR-181b-5p has been found also to be involved in various diseases such as glioblastoma [35], bladder cancer [36], prostate cancer [37], gastric cancer [38], and pancreatic cancer [39]. It is, therefore, non-specific miRNA, which makes it difficult to use it as a reliable unique biomarker for CC.

Small molecules interaction

Three out of 9 upregulated miRNAs in HeLa cells were found to interact with an array of small molecules including those used as chemotherapy. hsa-miR-30d-5p is a target of cisplatin, and this might be indicated with the validity of cisplatin in sanitizing CC to other chemotherapy [40]. Meanwhile, trastuzumab also might target hsa-miR-30d-5p, in the course of treating Her2-producing CC [41].

Nevertheless, 5-aza-cytidine found target hsa-miR-23b-3p in CC, although the mechanism of action of this interaction still not clear [42]. Furthermore, over expression of miR-23b-3p was found to sensitize HeLa cervix cells to 5-flourouracil [43], and that might indicate the effectiveness of using 5-FU to treat cervical cancer.

In this context, temozolomide might target hsa-miR-145-5p, especially in glioblastoma [44], and many other cancers [45,46].

Long non-coding RNA interaction

Long non-coding RNAs (lncRNA) function to modulate transcription factors via various mechanisms, including functioning themselves as co-regulators or enhancing/suppressing transcription factor activity. In this study, eight miRNAs (hsa-miR-20b-5p, hsa-miR-30c-5p, hsa-miR-181b-5p, hsa-miR-23b-3p, hsa-miR-30d-5p, hsa-miR-143-3p, hsa-miR-31-5p, and hsa-miR-145-5p) were found to interact with 253 lncRNAs. Examples include JRK, MAL2, SNHG1, MALAT1, and HCG18. JRK is highly expressed in patients with cancer, and it is regulated by several types of miRNA [46]. Likewise, Myelin and Lymphocyte Protein (MAL2) exerts tumor suppressor and an oncogene function in different cancers via regulating several miRNAs [47]. Meanwhile, mall nucleolar RNA host gene 1 (SNHG1) acts as a sponge of miR-145, and hence, regulate its function [48].

Metastasis associated lung adenocarcinoma transcript 1 (MALAT1) is a lncRNA upregulated in metastatic carcinoma cells, as it function to control alternative splicing and transcriptional reg-

ulation. It is regulated by hsa-miR-31-5p [49]. Furthermore, HCG18 binds to miR-146a-5p and downregulate its expression [50].

Conclusion

In the present study, 24 cancer-related miRNAs was profiled in cervical cancer cells (HeLa). Nine miRNAs were found to be upregulated (hsa-miR-31-5p, hsa-miR-23b-3p, hsa-miR-30d-5p, hsa-miR-206, hsa-miR-20b-5p, hsa-miR-30c-4p, and hsa-miR-145-5p). these upregulated miRNAs were uploaded to MiRnet network prediction online tool to predict the target genes, diseases, lncRNA, and small molecules. Data obtained revealed that the upregulated miRNA have different target categories; 8 miRNAs were found to target 2429 genes, 2 were found to target 20 different diseases, 3 were found to target 31 small molecules, and 8 were found to target 253 lncRNA. Data concluded that miR-20b-5p could be used as potential biomarker as it represents the most highly expressed miRNA profiled in CC cells.

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Conflict of Interest

The authors declare no conflict of interests.

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