miRNA, the inconspicuous but key player in the development of cervical cancer

Cervical cancer is among the top three cancers affecting women below 45 years in several countries. Both the alterations and modification in deoxyribonucleic acid (DNA) sequences play a critical role in the onset and progression of cervical cancer. The addition of a methyl group to one of the four nucleotide sequences in DNA, mainly cytosine, is responsible for governing the expression of genes. The altered and inappropriate expression of a gene is crucial for cancer initiation, development, and progression. Our cells contain two types of ribonucleic acids, and these are coding and non-coding. While the coding RNAs directly takes part in the protein synthesis, non-coding RNAs regulate the expression of coding RNAs. Many living organisms harbor a class of non-coding RNA called the microRNAs (miRNAs) to control protein-coding genes. Thus, the precise expression of miRNAs is important for fine-tuning the expression of protein-coding genes. Our study found that miRNAs and their target genes are abnormally expressed during cervical cancer conditions. The regulation of miRNA expression by DNA methylation levels can be used as early and reliable detection of cervical cancer. Early detection of cervical cancer improves the quality of women suffering from cervical cancer.

Fig. 1. Cervical cancer awareness.

We have identified the DNA methylation pattern by a technique called bisulfite DNA sequencing. The method involves using a small part of the cervical tissue from women and extracting the DNA

at different stages of cervical cancer progression. The sodium bisulfite treated DNA helps distinguish between the methylated and unmethylated DNA. The DNA when sequenced provides information at the single nucleotide level the modifications between a non-malignant and a malignant sample. Our study found that two miRNAs (miR-375 and miR-196a-1) whose activity was altered by cytosine methylation during the progression of cervical cancer. The increased methylation of miR-375 and miR-196a-1 showed over 80% sensitivity and 70 to 100% specificity to distinguish the tumor samples from normal samples. An inverse correlation between methylation and expression pattern was identified between miR-375 and miR196a-1. Additional studies showed that miR-375 and miR-196a-1 as DNA methylation regulated miRNAs.

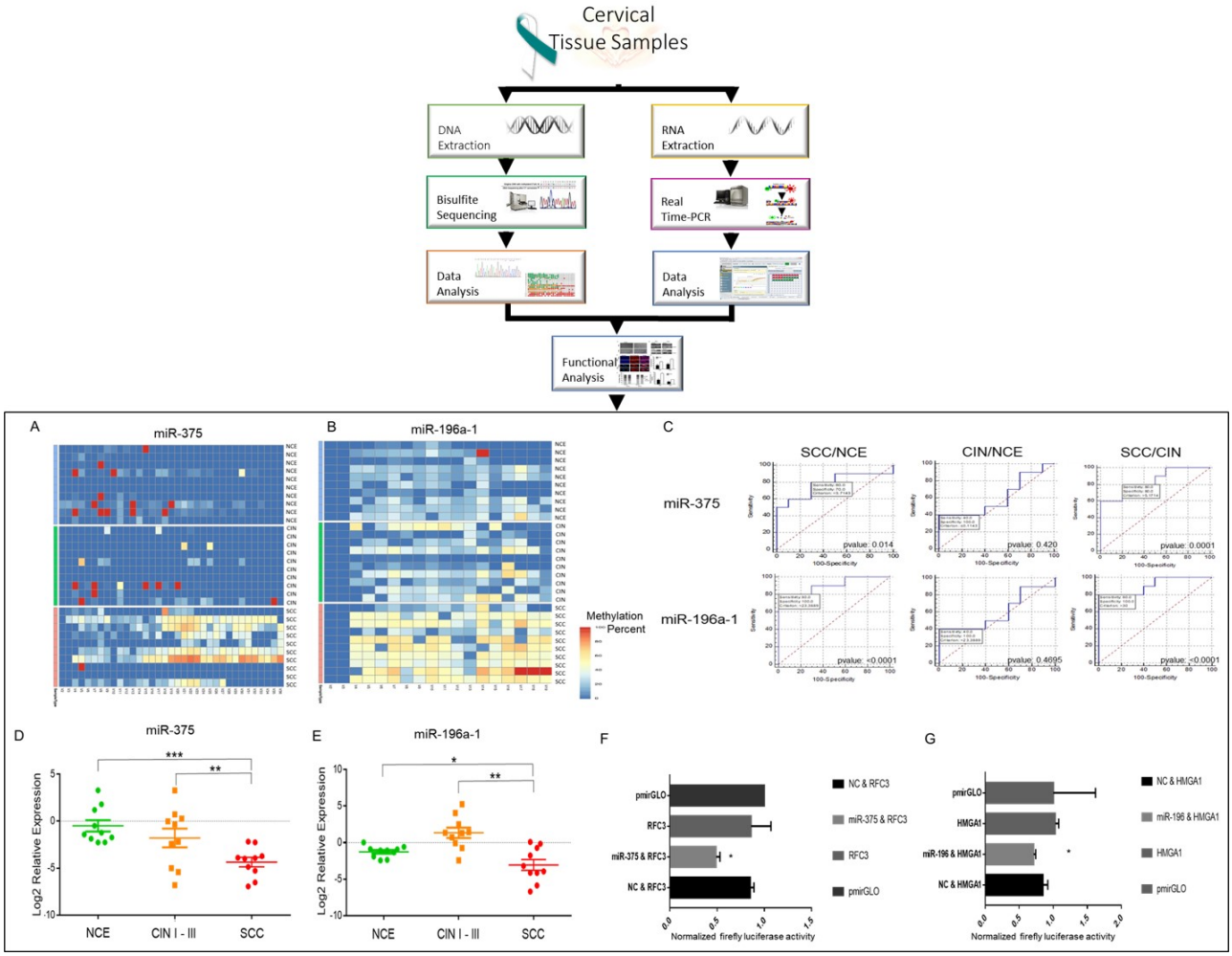

Fig. 2. An overview workflow of epigenetic factors involved in cervical cancer. DNA and RNA were extracted from the clinical samples followed by bisulfite DNA sequencing and real-time PCR to determine the methylation status and expression of miR-375 and miR-196a-1 respectively. Next, we performed functional analysis such as an effect on cell proliferation. Heat map of 2 differentially methylated miRNAs miR-375 (A), and miR-196a-1 (B) in 10 each normal, premalignant, and malignant samples. C) Sensitivity and specificity analysis of methylation level of miR-375 and

miR-196a in different groups (SCC/NCE, CIN/NCE, and SCC/CIN). Expression analysis of miR-375 (D) and miR-196a-1 (E) in clinical tissue samples. The miR-375 and miR-196a-1 mimics showed significant suppression of luciferase activity of the vector containing portions of RFC3 (F) and HMGA1 (G).

We have found that the DNA methylation caused the dysregulation of miRNA expressions and altered gene functions (*RFC3* and *HMGA1*). This led to altered pathways that affected the basic function of the cell. This small yet catastrophic chain of events assists the cell to grow uncontrollably and leads to cervical cancer's pathogenesis. Our study provides a framework for understanding the DNA methylation differences of miRNAs among individuals of different stages of cervical cancer and the potential of these miRNAs to better equip ourselves to fight cancer.

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