Research & Reviews on Cervical Cancer

Chapter 4

Role of Non Coding Sequences in Cervical Cancer

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1. Cervical Cancer

Cervical cancer (CC) is one of the most deadly yet easily preventable cancer types of women which is responsible for more than 530000 cases and 264000 deaths, annually worldwide. The occurrence of cervical cancer is reported to be more in women of developing countries such as in Latin America, sub-Saharan Africa, and Indian subcontinent [1,2]. Cervical cancers include malignancies of cervix, which is the narrow portion of the uterus where it joins with the vagina. There are two types of cells on the surface of the cervix namely, squamous and columnar and most cervical cancers originate from squamous cells. Cervical cancer usually develops in a low pace; beginning with a pre cancerous condition called dysplasia which can be detected by Pap smears and is assuredly curable at the early stage [3]. Human Papilloma Virus (HPV) infection is the primary cause for almost all cervical cancer incidences and the infection is mainly transmitted by sexual contacts. There are 15 oncogenic HPV virus strains and two among them viz, 16 and 18 are responsible for almost 75% of the cervical malignancies [4,5]. Other major risk factors include tobacco smoking, immunity compromising disease like HIV, regular intake of conception prevention pills, early age sex encounters, numerous sexual accomplices etc [6].

Like all other malignancies, CC is also caused due to deregulated cellular processes which culminate in carcinogenesis. Many different genes and their products contribute to the initiation and progression of CC. The role of non coding sequences are however less explored in CC. This chapter mainly focuses on discussing the role of non coding sequences as a contributory factor in CC.

1.1. Role of protein coding sequences in Cervical Cancer: Regulatory proteins

There are many proteins which show deregulated expression in cervical cancer including a histone H4 Lys-20 methyl transferase called SET8. SET8 is also known as KMT5A, SETD8 or PR-SET7 and is involved in the transfer of single methyl group to histone H4 lysine at position 20. Under normal physiological conditions SET8 has many intracellular roles including activation or repression of gene expression, sustaining chromosome structure and stability, managing DNA damage, regulating cell cycle and halting premature chromatin compaction in the S phase. Studies by Luan and Wang in 2016 have showed that SET8 is over expressed in cervical cancer tissues. SET8 knock down studies have indicated suppressed proliferation and metastasis ability in SiHa cell line and over expression studies in C4-1 cell line showed enhanced cell growth and metastasis [7]. Proliferating cell nuclear antigen (PCNA) is another protein associated with cervical cancer. PCNA protein is located within the nucleus and is a co factor for DNA polymerase delta. Under physiological conditions in response to DNA damage PCNA is ubiquitinated and plays eminent role in RAD6-dependent DNA repair pathway. PCNA is reported to be over expressed in many cancer types including cervical cancer where it promotes cell proliferation and tumorigenesis [8-10]. Protocadherin, PCDH10 is involved in the progression of cervical cancer where it is immensely prone to promoter hyper methylation. Insulin like growth factor-2 (IGF2) is another important protein which showed increased expression in cervical cancer when compared normal cervical tissues [11]. C-myc is an oncogenic transcription factor which regulates the expression of many miRNAs associated with cervical cancer [12,13]. PIWI sub family of Argonaute proteins have distinguished role in cancer pathology due to their unique ability to interact with piRNAs. Cervical cancer cells are characterized by the expression of PIWI proteins such as HIWI and HILI and the expression of which is associated with human papilloma virus infection. An elevated level of HIWI was associated with cancer invasion. HILI inhibits tumor suppressor P53 there by promotes cell proliferation and invasion [14-16]. Like in any other cancer type, pro angiogeic cytokine, VEGF and matrix metalloproeinase, MMP9 are over expressed in cervical carcinoma [20]. MMPs play eminent role in extracellular matrix (ECM) degradation during cancer cell migration and invasion as a part of metastasis. Urokinase-type plasminogen activator (uPA) is a serine protease that plays major role in the activation of MMPs and in the conversion of plasminogen to plasmin, in order to facilitate the free movement of cancer cells in the surrounding ECM [75]. uPA is reported to be over expressed in cervical carcinoma, hence could be developed as a potent biomarker for the diagnosis of cervical cancer [76]. PDCD4 is a recently identified tumor suppressor gene which is down regulated in different cancer types including in cervical cancer [77]. It has been suggested that PDCD4 binds to eukaryotic translation initiation factor 4A (eIF4A), which then regulate AP-1 and followed by MMPs [148,149]. Laminin-332, a heterotrimeric protein containing 3 chains such as LAMA3, LAMB3 and LAMC2, is considered as a marker for invasiveness in cervical lesions [78]. FOXO1 (Forkhead box protein O1),

a transcription factor which is over expressed in CC, could down regulate the expression of CDK inhibitors like p27 and p21 and up regulate cyclin D1 [64].

Ample literature is available related to the role of coding sequenced in CC, therefore in this chapter we will focus more on the role of non coding sequences in initiation and progression of CC.

1.2. Role of Non-coding Sequences of the Genome in Cervical Cancer: Regulatory RNAs

1.2.1. Role of long non coding RNAs in cervical cancer

Long non coding RNAs (lncRNAs) are endogeneous non coding transcripts of ~200 nucleotides long and were considered as transcriptional waste products in the past. Recent studies have demonstrated the unbeatable role of lncRNAs in different cellular events [69-71]. Aberrant expression lncRNA is reported to be associated with various pathological conditions including cancer [35,72-73].

1.2.1.1. Long non coding RNAs with oncogene function

Many lncRNAs have been implicated in CC. Let us first look into examples of lncRNAs that exhibit oncogenic effects. Cervical carcinoma high expressed 1 (CCHE1) is a lncRNA which shows abnormal expression in cervical cancer. CCHE1 is of 2500 nucleotide (nt) in size and the gene is located on chromosome 10. CCHE1 is reported to be up regulated in cervical cancer which in turn is associated with enlarged tumor size, advanced FIGO stage, invasion and hapless prognosis. CCHE1 regulates the expression of PCNA by binding on its mRNA there by promotes cell proliferation in cervical cancer [18].

lncRNA XLOC_006390 is yet another lncRNA which is up regulated in cervical cancer cell lines. XLOC_006390 could promote cell proliferation and migration by regulating SET8 expression in CC. The over expression of XLOC_006390 was associated with the advanced FIGO stage and lymphatic and distant metastasis of cervical carcinoma [7].

HOTAIR (HOX transcript antisense intergenic RNA) is a 2158 nt-long lncRNA which harbors the antisense strand of the HOXC gene cluster on chr12q13.13 [19]. HOTAIR could promote the expression of VEGF and MMP9 *in vitro* which in turn enhances the aggressiveness of cervical cancer cells in culture. Increased expression of HOTAIR was associated with poor prognosis and increased rate of recurrence of cervical cancer [20].

H19 is a 2300 nt-long lncRNA whose gene is located at chr11p15.5. It has got dual functions; one is to act as a lncRNA and the other is to play the role of the precursor for miR-675 [21,22]. Under physiological conditions, H19 is expressed only in embryos and not in adults.

But it is re-expressed in many of the cancer types thus calling it as an oncofetal gene [23]. H19 functions as an oncogene in cervical cancer [74].

Cancer up- regulated drug resistant (CUDR) is another feto oncogenic lncRNA similar to H19. It is a 2200 nt-long lncRNA which is located in the 19p13.1 chromosomal region. Under normal condition, CUDR is present only in fetal tissue apart from cardiac tissue [25]. It is re-expressed and up regulated in many cancer tissues including cervical cancer where it promotes resistance of cells towards cisplatin [26].

Colon cancer associated transcript 2 (CCAT2) is a 1752 nt-long lncRNA which induces chromosome instability, tumor progression and metastasis in many number of cancers including cervical cancer. CCAT2 gene is located on chromosome 8 at q24.21 position [29]. When CCAT2 was knocked down using siRNA in cervical cancer cells, it resulted in significant reduction in the proliferation and survival [30].

It has been reported that Antisense non coding RNA in the INK4 locus (ANRIL) enacts the role of an oncogene in cervical cancer. ANRIL inhibits p15 expression thereby facilitates cancer cell proliferation [31]. ANRIL is located at chr 9p21 region and is of 3800 nt-long. It epigenetically influences its neighboring tumor suppressors CDKN2A/B there by plays pivotal role in cell proliferation and senescence [32].

EZH2 binding long non coding RNA in cervical cancer (LncRNA EBIC) is 1500 nt in size and is located at 12q22 chromosomal region. LncRNA EBIC inhibits *E Cadherin* expression and there by promotes cell invasion in cervical cancer [33].

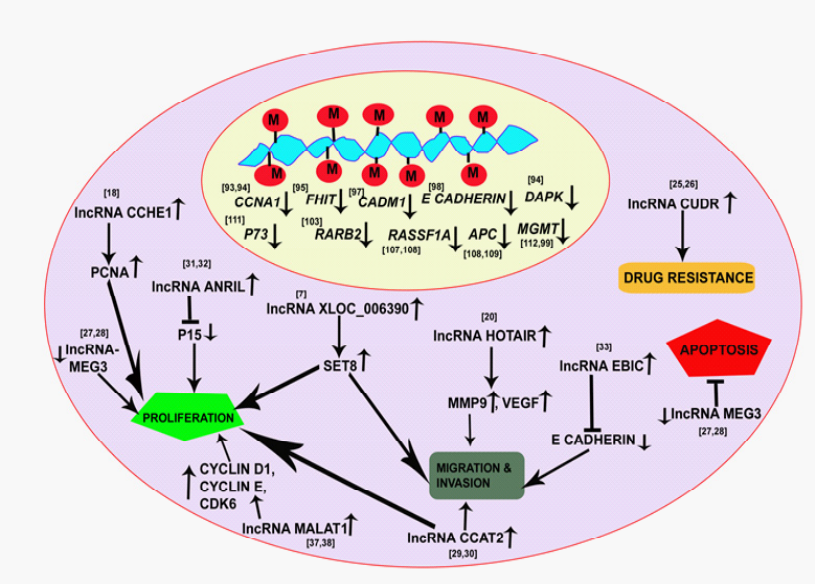

Figure 1: Schematically represents the major lncRNAs and their targets genes involved in the progression of CC. It also represents major tumor suppressor genes that undergo promoter hypermethylation in CC.

Metastasis-associated lung adenocarcinoma transcript-1 (MALAT-1) is yet another lncRNA associated with cervical cancer. It is an 8000nt long lncRNA which is located in

chr11q13.1 [37]. It has been reported by Zhang et al that MALAT-1 is over expressed in cervical cancer and the elevated expression of MALAT-1 was directly correlated with proliferation and metastasis of cervical cancer cells. When MALAT-1 was knocked down in cervical cancer cell line CaSki, there was a corresponding decrease in the expression of cyclin D1, cyclin E and CDK6, finally causing increased no. of cells in G1 phase [38].

1.2.1.2. Long non coding RNAs with tumor suppressor function

Unlike the oncogenic counter parts, these lncRNAs are responsible for reduction of cellular proliferation on tumor load. The example of tumor suppressor lncRNAs include, maternally expressed gene (MEG3) which is a 1600 nt-long lncRNA, generally down regulated in cervical cancer implying the tumor suppressive role of this lncRNA. MEG3 gene is located in the DLK1-MEG3 locus in the chr 14q32.3 [27]. MEG3 over expression has been reported to halt proliferation and cause increased apoptosis rate in cancer cells through different mechanisms either dependent or independent on p53 pathway [28].

Growth arrest specific transcript 5 (GAS5) is another lncRNA with tumor suppression properties. GAS5 is 651 nucleotides long and the gene is located in chromosome 1 at q25 locus [34]. Owing to its tumor suppresser action, GAS5 is reported to be down regulated in cervical cancer tissue. It has been demonstrated as an independent marker for predicting clinical outcome in cervical cancer patients though further studies are essential for the better understanding of its mechanism of action [17].

Table1: Represents major lncRNAs, their functions and effects of their actions in CC

Long Non Coding RNAs	Function	Effect	Reference
CCHE1	Oncogene Regulate the expression of PCNA	Enlarged tumor size, advanced FIGO stage, invasion and hapless prognosis. Promotes CC proliferation by upregulating PCNA.	[8-10,18]
XLOC_006390	Oncogene Regulate the expression of SET8	Advanced FIGO stage and lymphatic and distant metastasis of cervical carcinoma	[7]
HOTAIR	Promote the expression of VEGF and MMP9 in vitro	Improved aggressiveness, poor prognosis and increased rate of recurrence of cervical cancer	[20]
H19	Precursor for miR-675 Oncofetal gene	Oncogenic in CC	[21-23,74]
CUDR	Oncofetal gene, Oncogenic in CC	Resistance of CC cells towards cisplatin	[25,26]
CCAT2	Oncogenic	Induces chromosome instability, tumor progression and metastasis	[29,30]
ANRIL	Oncogenic, inhibits p15 expression, epigenetic modification of CDKN2A/B	Promotes cell proliferation	[31,32]

EBIC	Oncogenic, Inhibits E Cadherin	Promotes cell invasion	[33]
MEG3	Tumor suppressor, Down regulated in CC.	MEG3 over expression inhibited proliferation and induce apoptosis in different mechanisms either dependent or independent of p53.	[27,28]
GAS5	Tumor suppressor	Independent marker for predicting clinical outcome in cervical cancer patients	[17,34]
LET	Tumor suppressor	Biomarker for prognosis of CC	[36]

Low expression in tumor (LET) is a noval lncRNA with 2600 nt in size and is located on chromose 15, at q24.1. It is reported to be down regulated in a number of cancer types including cervical cancer. LET could be considered as an independent biomarker for prognosis in cervical cancer patients as there exist a relation between the LET expression and the clinic- pathological parameters of the patients. However further studies are required to substantiate the role of LET towards this direction [36]. **Table 1** and **Figure 1** respectively represents the list and scheme of major lncRNAs involved in CC.

1.2.2. Small non coding RNAs in cervical cancer

Apart from the lncRNAs, small RNA molecules can also regulate CC. microRNAs (miRNAs), the micro regulators of gene expression, occupy a wide range of organisms and biological systems. miRNAs are short, noncoding, single-strand RNA molecules that are transcribed as precursor molecules and are processed to mature miRNAs. miRNAs are loaded into the RNA induced silencing complex, which then recognizes sequences located mostly in the 3'untranslated regions (UTRs) of target mRNAs via imperfect base pairing, thereby regulating the expression of these genes predominantly by translational repression. The miRNAs with increased expression in tumors are thought to function as oncogenes and are called as oncomirs and they harbour complimentary binding sites for many of the tumor suppressor genes or genes those control cell differentiation or apoptosis [115].

1.2.2.1. miRNAs and piRNAs in cervical cancer

miRNAs are small, non coding RNAs having 22-25 nt long and are mainly involved in post transcriptional regulation of gene expression. miRNA genes harbor a variety of coding and non coding locations of the genome including intergenic, introns, exons, 3' UTRs and non-protein coding genes [39] . It was reported that ~40% of the reported miRNA genes occupy the intronic regions of the protein coding genes and ~10% on the lncRNA transcripts. miRNAs genes related to cervical cancer are predominantly located on intronic region and a few of them on the 3' UTRs [40]. Sharma et al in 2014, have suggested that most of the oncogenic miRNAs (oncomirs) associated with cervical cancer are located on the q arms of chromosome 1 and 17 [41].

Involvement of miRNAs in cervical cancer has been extensively studied in the recent years. Lee et al in 2008 have profiled the expression pattern of 157 human mature miRNAs in cervical cancer tumor biopsies. The study comprised 10 tumor biopsies and 10 normal tissues. From the study they have identified 70 miRNAs which were differentially expressed in cervical cancer (CC) tissues when compared to normal cervical tissues. Among the 70 miRNAs, 68 were upregulated and remaining 2 miRNAs were down regulated. They have short listed top ten over expressed miRNAs which included, miR-199s, miR-9, miR-199a*, miR-199a, miR-199b, miR-145, miR-133a, miR-133b, miR-214 and miR-127. miR-149 and miR-203 were the only miRNAs which were down regulated. Further they have mechanistically validated the role of miR-199a in cervical cancer by transfecting anti miR-199a in CC derived cell line and found that cell growth is getting retarded upon anti miR-199a over expression [42]. Later in 2010, Hu et al have checked the expression of 96 cancer related miRNAs in 102 CC tumor biopsy samples. The results from their study indicated that miR-200a and miR-9 are of prime importance as they regulate metastasis and metabolism respectively, in CC cells [43].

Similarly, Pereira et al have compared the expression profile of miRNAs between normal cervical tissues, moderate or severe dysplasia and invasive squamous cell carcinoma infected with HPV16. Their study have put forward a number of significant miRNAs, among them miR-16, miR-21, miR-106b, miR-135b, miR-141, miR-223, miR-301b and miR-449a were significantly over expressed in dysplasia as well as in squamous cell carcinoma tissues when compared to the normal tissues. They have also showed that miRNAs such as miR-21, miR-135b, miR-223 and miR-301b were significantly up regulated in squamous cell carcinoma tissue when compared to normal or dysplasia tissue, which in turn opened up a door to distinguish invasive squamous cell carcinoma and dysplasia tissues from normal tissue [44].

Li et al in 2011 have reported the importance of miR-100 in the progression of CC. They have showed that miR-100 gets down regulated as CC gets advanced. They have also suggested that down regulation in miR-100 expression would reflect on the expression of its target gene PLK1 (Polo like kinase 1), which would finally help the cancer cells to evade apoptosis and tune well to proliferation [45].

Cai et al have reported that members of miR-302-367 cluster could retard growth and development of tumor in cervical cancer where it down regulates the expression of cyclin D1 and AKT1 and promotes the expression of p27 and p21 proteins [46]. miR- 17-5p, a member of miR-17-92 cluster targets TP53INP1 and takes up the role of tumor suppressor in cervical cancer [47-49]. According to the reports by Huang et al minimal expression of miR-100 and miR-125b were associated with poor prognosis [50]. miR-497 was reported to be a tumor suppressor miRNA which could be utilized as a prognostic biomarker [51]. Similarly, miR-20a, a circulating miRNA, was reported as a potential biomarker for detecting lymph node metastasis in CC [52]. Some miRNAs like miR-106a and miR-16 showed differential expression as the

tumor progresses from its moderate to advanced stages. These miRNAs showed decreased expression in the initial stages of CC and as the stages get advanced their expression levels increased [41].

The altered miRNA expression in cervical cancer when compared to the normal tissue could be due to the chromosomal aberrations or epigenetic modifications. For instance, miR-944 is a cervical tissue specific miRNA whose chromosomal location 3q27-28 is reported to be amplified in cervical cancer [53,54]. Yao et al have found that hyper methylation of different miRNAs such as miR-1286, miR-432, miR-1290, miR-641, miR-1287 and miR-95 are associated with HPV infection in cervical carcinoma cell lines [55].

Chemotherapy is the main treatment strategy employed in cervical cancer. Developing resistance towards the chemotherapeutic drug is the main hurdle with chemotherapy in which miRNAs play vital roles. Reports from various studies have showed that ectopic expression of different miRNAs including miR-15b, miR-16 [56], miR-218 [57], miR-214 [58] and miR-155 [59] have reduced the resistance of cancer cells towards cisplatin. Recent reports from our lab showed that exosomal miRNAs, miR106a & b derived from cisplatin resistant hepato carcinoma cells could alter the sensitivity of cervical cancer cells (Hela) towards cisplatin, where miR-106a & b have got direct regulatory effect on a lysine deacetylase enzyme, SIRT1 [114].

Evading apoptosis is considered as one of the important survival strategies of cancer cells which involve miRNAs with their unique roles. Liu et al have proved that miR-143 has got binding site on the 3'UTR of an anti apoptotic gene, Bcl2. Over expression of miR-143 induced apoptosis in Hela cell line and the effect was reverse when the cells were transfected with anti-miR-143 [60]. Nevertheless miR-886-5p was reported to be over expressed in cervical cancer tissue where it targets a pro apoptotic gene Bax [61]. Yao et al in 2009 have reported that miR-21 is over expressed in cervical cancer and it targets a tumor suppressor gene namely programmed cell death 4 (PDCD4) in Hela cell line [62].

miR-424 is reported to target Chk1 (checkpoint kinase 1) and p-Chk1 in cervical cancer cells. Xu et al have demonstrated that over expression of miR-424 down regulates the expression of Chk1. Further, Chk1 knock down studies have showed that decrease in the expression of Chk1 is positively co related with the expression of Matrix metalloproteinase9 (MMP9) [63]. FOXO1 (Forkhead box protein O1) is targeted by miR-223 [64] and miR-182 [65] and these miRNAs are down regulated in cervical cancer.

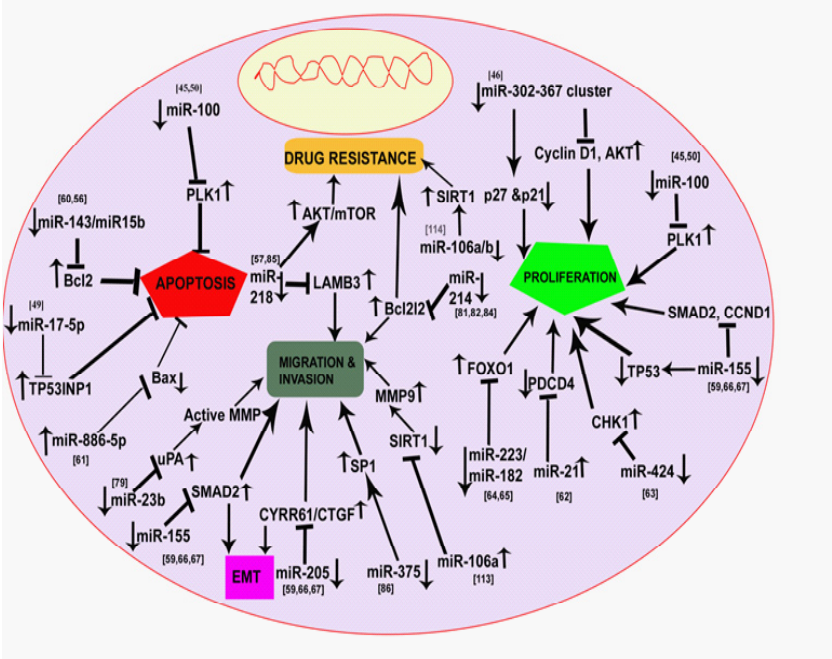

Figure 2: Schematically represents the major miRNAs and their targets genes involved in the progression of CC.

It has been reported by Yeung et al that the oncogenic HPV-16 E6 protein could down regulate expression of miR23b which in turn could facilitate the over expression of its target gene uPA. uPA induces MMP activation and thereby promotes migration in cervical cell carcinoma [79]. Reports from our lab suggested that miR106a could regulate the expression and activity of MMP9 in a SIRT1 dependent mechanism in Hela cell line [113]. Peng et al have reported that miR-214 is down regulated in cervical cell carcinoma and its expression negatively affects the growth of cervical cells. They have demonstrated that miR-214 targets GALNT7 (UDP-N-acetyl-α-D-galactosamine: polypeptide N-acetylgalactosaminyltransferase 7) and diminishes its expression [80]. GALNT7 catalyses the initiation step of O-glycosylation and uncontrolled glycosylation is the hall mark of oncogenic transformation, invasion and metastasis [81]. Similarly, Qiang et al have reported that miR-214 is down regulated in cervical cancer and it targets plexin-B1 gene [82]. Plexin B1binds to Sema4D/CD100 [83] which causes the recruitment of active Rac proteins and finally it holds back Rac from activating PAK resulting in the disassembly of cytoskeletal structures [84].

miR-218 is found to be down regulated in cervical squamous cell carcinoma with a resultant increase in the expression of its target protein LAMB3 which promotes cancer cell migration and invasion [85]. Wang et al have showed that miR-375 targets the 3'UTR of SP1 [86], where SP1 is a link between MAPKinase (Mitogen-activated protein kinase) pathway and VEGF (Vascular endothelial growth factor) expression [87]. In addition to that, elevated miR-375 suppressed cell proliferation by halting G1-S transition in cervical cancer cells and SP1 knockdown inhibiting cell migration and invasion [86]. VEGF being a potent cytokine that induces angiogenesis have been extensively studied in many cancer types and is known to be regulated by many miRNAs [88]. So, the study towards the regulatory role of miRNAs in VEGF expression during cervical carcinoma progression will be highly appreciated.

Table 2: Represents major small RNAs, their functions and effects of their actions in CC

Non Coding sequence	Function	Effect	Reference
miR-199a*	Over expressed in CC	Promotes cell growth in CC cells	[42]
miR-100	Down regulated in CC, Targets PLK1	Evades apoptosis, Induces proliferation, Poor prognosis	[45,50]
miR-17-5p	Targets TP53INP1	When ectopically over expressed Suppresses tumor growth and promoted apoptosis in CC	[49]
miR-302-367 cluster	Targets Cyclin D1 & AKT, Promotes p27 & p21 expression	Suppresses cell proliferation in CC	[46]
miR-125b	Down regulated in Small cell cervical carcinoma	Poor prognosis	[50]
miR-497	Targets IGF-1R	Potential prognostic biomarker	[51]
miR-20a	Circulating miRNA , Over expressed in CC	Potential biomarker for detecting lymph node status in CC	[52]
miR-106a	Targets SIRT1 in Hela Cell line	Regulate the expression of MMP9 in a SIRT1 dependent mechanism. Enhances the sensitivity of Hela cells to cisplatin in a SIRT1 dependent mechanism.	[113,114]
miR-16	Different expression pattern at different stages of CC. Targets Bcl2	Down regulation of NDRG2 sensitizes Hela to cisplatin	[41, 56]
miR-944	Up regulated in CC	miR-944 chromosome location 3q27-28 is amplified in CC	[53,54]
miR-155	Tumor suppressor in Caski cell line.	Ectopic over expression in Caski cells negatively regulated EGF induced EMT, migration, invasion and proliferation also increased chemo sensitivity to DDP. Induced TP53 expression but reduced SMAD2 and CCND1 expression.	[59]
miR-214	Targets Bcl2l2 in Hela and C-33A cells. miR-214 expression is regulated by DNA methylation and histone deacetylation in CC. Targets GALNT7 Targets Plexin B1	Reduced expression of miR-214 in CC caused Inhibition of cell growth, invasion and migration. Ectopic expression in Hela and C-33A cell line induced apoptosis and chemo sensitivity to cisplatin. Promotes O-Glycosylation. Plexin B1- Sema4D/CD100 complex Inhibits Rac from activating PAK resulting in disassembly of cyto skeletal structures	[58, 81,82,84]
miR-143	Down regulated in CC Targets Bcl2	Induce Apoptosis	[60]
miR-886-5p	Over expressed in CC, Targets Bax	Induce Apoptosis	[61]
miR-21	Over expressed in CC, Targets PDCD4	Promotes cell proliferation	[62]
miR-424	Down regulated in CC, Targets Chk1	Promotes CC progression through increased Chk1 expression	[63]

miR-223	Down regulated in Hela cell line. Targets FOXO1	Promotes cell proliferation. FOXO1, pFOXO1, Cyclin D1/p21/p27 levels increases	[64]
miR-182	Down regulated in CC, Targets FOXO1	Active FOXO1 down regulates p27 & p21 and up regulates cyclin D1	[65]
miR-23b	Down regulated in CC MMP activation and enhanced migration	MMP activation and enhanced migration	[79]
miR-218	Down regulated in CC Targets LAMB3	miR-218 down regulation promotes cell migration and invasion in CC. When ectopically over expressed in Hela, miR-218 inhibited cell proliferation and induced chemo sensitivity to cisplatin by blocking AKT/mTOR signaling pathway.	[57,85]
miR-375	Targets SP1	Down regulation of miR-375 suppressed cell migration and invasion in cervical squamous cell carcinoma	[86]
miR-205	Down regulated in CC, Targets CYR61/CTGF	Promotes EMT, cell migration and invasion	[59,66,67]
miR-155	Down regulated in CC, Targets SMAD2	Promotes EMT, cell migration and invasion	[59,66,67]
piR-651	Up regulated in CC	Not available	[68]
miR-15b	Target s Bcl2	Down regulation of NDRG2 sensitizes Hela to cisplatin	[56]

miR-205 and miR-155 are down regulated in cervical cancer tissues. The target proteins of miR-205 and miR-155 respectively include CYR61/CTGF and SMAD2 [66,67,59]. When these miRNAs are over expressed in cervical cancer cells they negatively regulated EMT and inhibited cell migration and invasion [59]. In addition to miRNAs, Piwi interacting RNAs also show deregulated expression in cervical cancer. For example, piR-651 was up regulated in many cancer types including cervical cancer [68]. Although extensive literature is not available on the role of Piwi interacting RNAs in CC, it is expected that in near future its role will be clear owing to the volume of research involved now. **Table 2** and **Figure 2** respectively represents the list and schematic representation of major miRNAs involved in CC.

1.3. Role of promoter sequences in cervical cancer

Promoter sequences constitute the critical non coding part of the genome. A promoter is the regulatory sequence located a few base pairs up stream of a gene which is identified by different transcription factors in order to initiate transcription. Tumor suppressor genes (TSGs) are generally silenced in many numbers of cancers by epigenetic alterations of the promoter sequences, popular one among them being CpG methylation [89]. Most of the genes in the mammalian genome possess CpG islands in the promoter region. CpG island hyper methyla-

tion is a common epigenetic modification associated with TSG silencing in cervical carcinomas [90,91].

1.3.1. Epigenetic modification of promoter sequences of cell cycle associated genes in cervical cancer

p16 protein is a cell cycle dependent kinase inhibitor and is down regulated in most of the malignancies. However, p16 is over expressed in HPV mediated cervical carcinoma due to the influence of HPV. In CC, HPV E7 proteins interact with cellular pRb with a subsequent reduction in the free Rb followed by an increase in the expression of p16. P16 over expression in CC is associated with the severity of cervical neoplasia. However p16 promoter hyper methylation is commonly observed in cervical cancer progression [92].

Cyclin A1 is a cell cycle regulatory protein that binds to CDK2 and CDC2 kinases and is encoded by *CCNA1 gene*. It interacts with different other proteins including the members of Rb protein family, E2F-1 transcription factor and the proteins of p21 family. *CCNA1* methylation is reported to be common in cervical cancer. *CCNA1* is rarely methylated in normal cervical tissue and the percentage of *CCNA1*promoter methylation increases as the severity of the tumor progresses. Hence, *CCNA1* methylation is considered as a potent biomarker to distinguish normal or early stage neoplasia from the severe invasive cancer [93,94].

FHIT (Fragile Histidine Triad) is yet another protein associated with cell cycle regulation and apoptosis. Epigenetic silencing of FHIT is observed in cervical cancer cells by promoter methylation [95].

1.3.2. Epigenetic modification of promoter sequences of cell adhesion associated genes in cervical cancer

CADM1 (Cell adhesion molecule1) mediates epithelial cell adhesion and the loss of its function is associated with a number of cancers. CADM1 expression is epigenetically silenced by promoter methylation in cervical carcinoma and the frequency and density of methylation is proportional to the severity of cervical dysplasia associated with high risk HPV infection [96]. Recent studies have showed that *CADM1* promoter methylation could be utilized as an important biomarker for identifying women with high risk HPV infection heading towards high grade cervical dysplasia [97].

E Cadherin is a Ca^{2+} dependent cell adhesion protein whose expression is been silenced in different cancer types including cervical cancer. *E Cadherin* promoter methylation is not observed in normal cervical tissues and the frequency of methylation increases proportionally with the advancement in CC [98].

1.3.3. Epigenetic modification of promoter sequences of apoptosis associated genes in cervical cancer.

DAPK, a pro-apoptotic serine/threonine kinase, is down regulated in cervical cancer though promoter methylation of *DAPK* gene [99,100] and this epigenetic modification was detected in the plasma samples of individuals with cervical cancer [101,102]. Like in case of *E Cadherin* promoter methylation, the frequency of methylation on *DAPK* promoter is increased with the severity of cervical dysplasia [94].

p73 is a homologous protein of p53 thus comes under the *p53* tumor suppressor gene family. Though *p73* mutation is rare in human cancer as compared to *p53* mutations, it has been reported that promoter hyper methylation is present in 38.8% of cervical cancers with a corresponding reduction in the expression of *p73*. Moreover this epigenetic change was more evident in radioresistant cancers (58.0%) when compared to radiosensitive cancers (20.8%) [111].

1.3.4. Epigenetic modification of promoter sequences of cell signaling associated genes in cervical cancer.

RARβ2 (Retinoic acid receptor - β2) is a tumor suppressor gene and a member of the nuclear receptor super family. Epigenetic suppression of RARβ2 expression by promoter hyper methylation has been reported [103]. It has been reported by Jha et al that promoter demethylation in cervical cancer cell line like inSiHa and HeLa using natural compounds like curcumin and genistein led to the re-expression of RARβ2 [104].

RASSF1A (Ras Association Domain Family 1 isoform A) is a tumor suppressor gene associated with various cellular regulatory events including apoptosis, cell cycle arrest etc. Many epithelial tumors are characterized by the reduced expression of RASSF1A, where the gene is silenced by promoter hypermethylation [105]. However only less than 10% of the cervical squamous cell carcinoma were found to possess *RASSF1A*promoter hyper methylation. None of the HPV16/18-positive squamous cell carcinoma possessed RASSF1A promoter hyper methylation. Where as 20- 45% of the adenocarcinomas and adenosquamous carcinomas exhibited *RASSF1A* promoter hyper methylation [106,107].

APC (Adenomatous polyposis coli) is a tumor suppressor protein involved in Wnt/β-catenine pathway. Reduced expression of APC in cancer is responsible for the over expression of β-catenine target genes including c-myc, cyclin D, Caspases and ephrins [108]. APC gene silencing by hypermethylation was observed morec frequently in cervical adenocarcinoma when compared to squamous cell carcinoma [109]. APC promoter hyper methylation was observed in vitro in HeLa cell line (cervical adeno carcinoma cell line) and not in SiHa (cervical squamous carcinoma cell line). In vitro demethylation experiments in HeLa cells caused re-

expression of APC [110].

1.3.5. Epigenetic modification of promoter sequences of MGMT (A DNA repair protein)

MGMT (O^6-methylguanine-DNA methyltransferse) is a DNA repair protein whose expression is reduced in approximately 17% of the cervical carcinomas due to DNA hypermethylation or deacetylation [112]. Kim et al in 2010 have reported that the frequency of *MGMT* promoter hyper methylation increases severity verity of cervical cancer [99]. **Table 3** and **Figure 1** respectively represents the list and scheme of major gene promoters involved in CC.

Table 3 : Represents major gene promoters, their functions and effects of their actions in CC

Hyper methylated Promoter in CC	Function	Effect	Reference
P16	Cell cycle dependent kinase inhibitor. Promotes cell cycle arrest in normal cell	*P16* promoter hypermethylation is commonly observed in CC.	[92]
CCNA1	Binds to CDK2, CDC2, E2F1, Rb family proteins and p21 family proteins. Tumor suppressor	*CCNA1*promoter methylation can be used as a biomarker to distinguish between early and late stages of CC.	[93,94]
FHIT	Tumor suppressor.	Not available	[95]
CADM1	Mediates epithelial cell adhesion.	Could be developed as a biomarker to identify women with high risk HPV infection leading towards high grade CC.	[97]
E Cadherin	Cell adhesion	The frequency of E Cadherin promotermethylation is increased with the severity of cervical dysplasia	[98]
DAPK	Induces apoptosis under normal physiological conditions	The frequency of *DAPK* promoter methylation is increased with the severity of cervical dysplasia	[94]
P73	Homologous of p53	Promoter methylation is more evident in radioresistant cancers	[111]
RARβ2	Associated with cell growth suppression function of retinoic acid	Tumor progression	[103]
RASSF1A	Cell cycle suppression, apoptosis and genetic instability	Cell cycle progression, Reduced apoptosis	[107,108]
APC	Involved in Wnt/β-catenine pathway	Reduced APC expression causes increase in the expression of c-myc, cyclin D, Caspases and ephrins	[108,109]
MGMT	DNA repair protein, protects genome from mutagens	The frequency of *MGMT* promoter hyper methylation increases with the severity of cervical cancer	[112,99]

To conclude, most of the tumor suppressor genes are epigenetically repressed by promoter hyper methylation in cervical cancer owing to the importance of promoter sequence (a non coding portion of the genome) in the development and progression of cervical cancer. Non toxic demethylating agents could be used in cancer therapy in order to bringout epigenetic alterations on the promoter sequences and thereby to facilitate the re-expression the of tumor suppressor genes in the cancer tissues. It therefore appears that like the coding sequences, the non-coding sequences are also important contributing factors in the development and progression of CC. These non-coding sequences indirectly affect the coded products to bring about its effect. The regulatory role of these sequences though identified and appreciated, a lot of work is pending before we get a holistic picture of their role in CC.

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