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Mini Review

# Recent Advances on the Molecular Mechanism of Cervical Carcinogenesis Based on Systems Biology Technologies

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ABSTRACT

Cervical cancer is one of the common malignancies in women worldwide. Exploration of pathogenesis and molecular mechanism of cervical cancer is pivotal for development of effective treatment for this disease. Recently, systems biology approaches based on high-throughput technologies have been carried out to investigate the expression of some genes and proteins in genomics, transcriptomics, proteomics, and metabonomics of cervical cancer. Compared with traditional methods, systems biology technology has been shown to provide large of information regarding prognostic biomarkers and therapeutic targets for cervical cancer. These molecular signatures from system biology technology could be useful to understand the molecular mechanisms of cervical cancer development and progression, and help physicians to design targeted therapeutic strategies for patients with cervical cancer.



**Contents**



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## 1. Introduction

Cervical cancer is the fourth most commonly diagnosed cancer in females behind breast, colorectal and lung cancers worldwide [1]. Among females, cervical cancer also ranks the fourth for cancer-related death in the world. According to estimate numbers from the WHO (World health organization), there are 570,000 new cases of cervical cancer, and about 311,000 deaths due to this deadly disease worldwide in 2018 [1]. In the United States this year, there are an estimated 13,240 cases and 4170 deaths from cervical cancer [2]. In China, 98,900 new cases of cervical cancer and 30,500 deaths are reported in 2015 [3]. In recent years, there is a common trend that most patients with cervical cancer died with unmeasurable pain and suffered at relatively young age [4]. Although the incidence rate of cervical cancer was declined due to increased screening in women and higher uptake of the HPV (human papillomavirus) vaccination in the developed countries, it is still one of the leading causes of cancer morbidity among females in less developed countries [1]. The five-year survival rate for patients with cervical cancer is less than 50% in many underdeveloped countries [5]. Moreover, the survival rate of cervical cancer patients at advanced stages is also low [6]. Thus, it is no exaggeration to note that exploration of molecular mechanism of cervical carcinogenesis is pivotal to obtain better treatment outcomes for cervical cancer patients. (See Fig. 1.)

Evidence has revealed that many factors have been found to be associated with cervical cancer development, such as smoking, immunesuppression, oral contraceptive use, high parity (a multiple number of pregnancies), and HPV infection [1]. It is widely accepted that HPV is the virtually important cause of cervical cancer development, although the pathogenesis of cervical carcinoma is complex. The cervical tumorigenesis is often initiated by persistent infection of high-risk HPV types, particularly virus types 16 and 18 [7]. More than 100 types of HPV have been identified and the viral typing plays a key role in determining the prognosis of cervical cancer [8]. Due to the different pathogenicities of HPV, it can be divided into low-risk virus types and high-risk types. Epidemiologic evidence showed that HPV16 and HPV18 are correlated with cervical cancer [9]. It is important to mention that not all cases with high-risk HPV infection will result in the high-grade cervical intraepithelial neoplasia (CIN) development and cervical cancer, suggesting that HPV infection is not sufficient to cause cervical cancer [10]. Most of the subclinical changes are transient because HPV infection is cleared spontaneously by the immune system. Only a minority of HPV infections lead to integration into the host genome, resulting in abnormal gene structures and functions and malignant transformation of cervical cells [10,11]. Genome-wide association studies (GWAS) have shown that cervical cancer has genetic variations in several susceptibility loci [12].

Accumulated evidence has demonstrated the reasons by which HPV infection causes carcinogenesis [13]. Two viral oncoproteins E6 and E7 could play a key role in the HPV-infected cervical cancers. When the viral genome integrates into the host DNA genome, E6 and E7 will be upregulated and subsequently deregulate critical proteins in cellular signaling pathways, such as inhibition of two important tumor suppressor proteins, p53 and pRb [14]. Lau et al. reported that DNA tumor virus oncogenes including E7 could bind and suppress the cGAS-STING DNA-sensing pathway [15]. It has been documented that not all integrations necessarily depend on the E6 and E7 oncogenes expression [16]. Notably, several reports have showed that cervical cancer has driver mutations such as PIK3CA (phosphatidylinositide 3-kinases catalytic subunit α), a key protein in the PI3K pathway, KRAS (Kirsten rat sarcoma viral oncogene homolog), and EGFR (epidermal growth factor receptor) [17]. Currently, clinical treatment managements for cervical cancer typically include surgery, radiotherapy, and platinum-based chemotherapy [18]. Treatment for early stage disease often is surgical therapy such as cervical conization, total simple hysterectomy, or radical hysterectomy based on extent of spread of cervical cancer [18]. Radiotherapy plays a pivotal role not only in locally advanced cervical cancer but also in postoperative therapy to prevent locoregional recurrence as an adjuvant therapy [18,19]. In the recent years, a series of systemic treatments, for instance, the platinum-based chemotherapy and the recent FDA approved pembrolizumab, have applied for recurrent and advanced cervical cancer [20]. Nowadays, the standard frontline chemotherapeutic treatment for cervical cancer is the combination therapy of carboplatin, paclitaxel, and bevacizumab [21]. Although screening and advanced therapeutic strategies have improved the survival rate of cervical cancer, some patients still die due to metastasis and drug resistance. Without a doubt, HPV vaccination could prevent the development of cervical cancer; however, many patients in underdeveloped countries cannot get HPV vaccination due to economic condition. Thus, it is pivotal to further understand the molecular mechanisms of cervical cancer development and progression, to discover the novel molecular diagnostic methods and systemic managements for cervical cancer. To achieve this goal, system biology approaches would

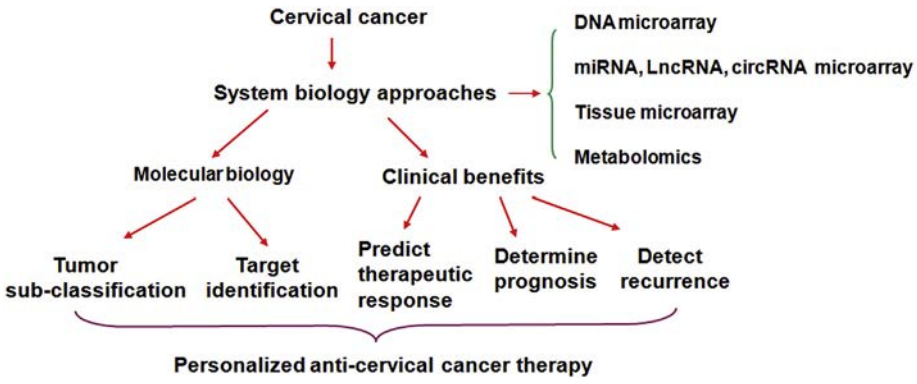


**Fig. 1.** Systems biology approaches are utilized in personalized medicine of cervical cancer. Systems biology approaches such as DNA microarray, tissue microarray, microRNA, lncRNA and circRNA microarrays could be applied to various areas in the clinical management including cervical cancer screening, diagnosis, detecting recurrence, and predicting therapeutic response.

be new tools to be applied. In the following sections, we will review the recent advance on the mechanism of cervical carcinogenesis by system and network biology, including genomics, transcriptomics, proteomics, and metabonomics.

## 2. Systems Biology Approaches in Cervical Cancer

Systematic biology technology is of great value due to its comprehensive, accurate and sensitive characteristics, which integrates various biological levels involved in genes, molecules, cells, organs and the environment. Systems biology approaches determine the mechanisms underlying certain conditions to dissect the dynamic changes and interactions among individuals [22]. As a new research tool, systems biology approach has been applied for the diagnosis and the discovery of new biomarkers on the platform of genomics, proteomics and metabolomics for diseases. Nowadays, systems biology has becoming a promising standard framework to explore the molecular mechanism of development and progression of human diseases including cancer [23]. These approaches including genomics, transcriptomics, proteomics and metabolomics have been performed to analyze the genome (DNA), transcriptome (mRNA), proteome (proteins), and metabolome (metabolites), respectively, in the development and progression of cervical cancer [24]. These system biology methods could be used for the prediction of prognosis and treatment outcomes in cervical carcinoma, which could have potential clinical applications for cervical cancer (See Fig. 1).

### 2.1. Genomics

The application of genomics in cervical cancer mainly measures the changes of oncogene and tumor suppressive gene profiles at the DNA level. Moreover, this assay is helpful to determine the correlations between gene expression changes and pathological features. Therefore, the application of genomics can provide a more comprehensive understanding of the mechanism of cervical cancer development and progression, and discover the biomarkers for cervical carcinoma. DNA chip technology is the most widely used in genomics analysis. DNA chip, also known as gene chip or DNA microarray, is based on the principle of complementarities, using densely arranged DNA probes to extract DNA or RNA information, and compare with the changes of gene expressions in different physiological states or diseases including cervical cancer.

#### 2.1.1. DNA Microarray

Wong et al. used DNA microarray that contained approximately 11,000 features to examine the expression profiles of cervical cancer compared with normal cervical tissues [25]. This study reported that about 40 genes were significantly different between cervical cancer and normal tissues, which can completely segregate between tumor tissues and normal samples. Moreover, clinical stage IB and IIB tumors were also be classified according to the different expression signatures. Furthermore, tumor samples that were responded to radiotherapy were also identified by expression patterns in cervical cancer [25]. This study suggests that DNA microarray might be useful for determining disease stages and predicting radiotherapy response in cervical cancer. Song et al. further analyzed the gene expression patterns among normal cervix, carcinoma in situ (CIS), and invasive cervical cancer using DNA microarray [26]. Among 15,286 genes, 458 genes were upregulated or downregulated compared with each other group. Upregulation of 22 genes and down-regulation of 40 genes were observed in invasive cervical cancer patients compared with CIS [26]. Moreover, the expression of several genes is associated with invasive cervical cancers including upregulation of RBP1 (Retinol binding protein 1), TFRC (transferrin receptor), SPP1 (osteopontin), SAA1 (serum amyloid A1), ARHGAP8 (Rho GTPase-activating protein 8), and NDRG1 (N-myc downstream-regulated gene 1), downregulation of GATA3 (GATA-binding protein 3), PLAGL1 (pleiomorphic adenoma gene-like 1), APOD (apolipoprotein D), DUSP1 (dual specificity phosphatase 1), and CYR61 (cysteine-rich, angiogenic inducer, 61) [26]. Zhu et al. reported that 1326 genes were upregulated and 1432 genes were downregulated in cervical cancer compared with normal cervix using oligonucleotide microarrays [27]. Among these genes, there are multiple upregulated genes, which are related with the apoptosis pathways, including Bcl-2 (B-cell lymphoma-2), Bcl-xL (B-cell lymphoma-extra large), and c-IAP1 (cellular inhibitor of apoptosis protein 1) in late-stage cancer compared to early-stage cervical cancer [27]. Consistently, 2036 differentially expressed genes were identified by whole genome microarray between cervical squamous carcinoma and normal cervical tissues, including 1282 downregulated genes and 754 upregulated genes. Notably, PDGFRA (platelet-derived growth factor-A), CAV1 (caveolin 1), and GJA-1 were confirmed to be important genes for invasion and metastasis in cervical cancer [28]. Similarly, 7530 significantly overexpressed genes were identified by a transcriptome analysis in locally advanced cervical cancer patients, which were involved in 93 dysregulated signaling pathways [29].

Using alignment of DNA microarray data, chromosomal alterations were identified to play an important role in the development of CIN and invasive cervical carcinoma [30]. Gain of 3q and loss of 4q were detected from invasion cancer cDNA arrays, indicating that alignment of microarray data by chromosomes might be useful to estimate chromosomal region aberrations [30]. DNA microarray has been used to identify the gene expression profile between chemoradiotherapy resistant and sensitive patients in advanced uterine cervical squamous cell carcinoma [31]. Specifically, 108 genes were validated to be differentially expressed between chemoradiotherapy resistant and sensitive patients. PDGFRα (platelet-derived growth factor receptor alpha) and PRKAR1A (protein kinase A type 1A) were increased in the chemoradiosensitive patients, whereas LDHA (lactate dehydrogenase A), SMUG1 (single strand selective monofunctional uracil DNA glycosylase 1), BAK1 (Bcl-2 antagonist killer 1), CDK7 (cyclin dependent kinase 7), BNIP3 (Bcl2 adenovirus E1B 19 kDa interacting protein 3) expressions were increased in the chemo-radiotherapy resistant patients [31]. A study described 22 upregulated and 181 downregulated genes, which response to radiotherapy and chemo-radiotherapy in cervical cancer patients using microarray gene expression profiling [32]. Another study identified potential transcriptional regulation of cervix cancer using microarray gene expression data and promoter sequence analysis of a curated gene set. The results revealed that E2F (adenovirus E2 factor) could have diagnostic/prognostic value and can be a potential therapeutic target in cervical cancer [33]. Lee et al. reported that Dkk3 (Dickkopf-Related Protein 3) was downregulated and played a role as a negative regulator of β-catenin in cervical cancer [34]. Several genes including p16Ink4a, MCM3 (minichromosome maintenance complex component 3), MCM5 minichromosome maintenance complex component 5), CDC6 (cell division cycle 6), Geminin, Cyclins A-D, TOPO2A (Topoisomerase 2α), CDCA1 (cell division cycle associated 1), and BIRC5 (baculoviral inhibitor of apoptosis repeat-containing 5) were identified to be differentially expressed by microarray analysis in cervical cancer [35]. Similarly, microarray expression analysis was utilized to identify expression profiles of candidate genes that distinguished squamous cell carcinoma and adenosquamous carcinoma [36]. This study provides evidence that specific genes could be used as biomarkers for prognosis and therapy targets in different clusters of cervical cancer. A DNA microarray and gene pathway analysis dissected that inactivation of DP1 induced p53 mRNA and increased p21Waf1/Cip1 and promoted senescence in cervical cancer cells [37]. Overexpression of LAP2α (lamina-associated polypeptide 2 alpha) was found to be associated with aberrant E2F and p53 activities by microarray, qRT-PCR and immunofluorescence analyses in cervical cancer [38].

#### 2.1.2. DNA Microarray for Determining Mechanism of HPV Infection

It has been reported that a DNA microarray-based method was used to detect infection and typing of HPVs [39]. Although HPV16 and HPV18

are observed in a majority of invasive cervical cancer, HPV18 infection is correlated with a more aggressive form of cervical cancer than HPV16 positive patients. To determine the mechanism of two types of HPV infection, DNA microarray was used and found that some genes were differentially expressed between HPV16- and HPV18-infected samples. This study identified that different genes involved in signaling pathways could serve a different role in HPV16- and HPV18- transformed cells [40]. In addition, HPV DNA testing by a DNA microarray chip has been used for primary screening for cervical lesions in Japan [41]. This report reveals that HPV DNA testing in combination with cytology is superior for CIN screening [41]. DNA microarray was used to identify and characterize potential markers for screening or treatment targets between 43 HPV16-positive cervical cancers and 12 healthy cervical epitheliums. It was found that 997 of 8638 genes were deregulated, including 6 upregulated genes CCNB2 (cyclin B2), CDC20 (cell division cycle 20), PRC1 (Protein Regulator of cytokinesis 1), SYCP2 (SC protein-2), NUSAP1 (nucleolar-spindle-associated protein 1), CDKN3 (Cyclin-dependent kinase inhibitor 3) that belong to the mitosis pathway [42]. Moreover, CDKN3 was validated to be a survival marker and a potential treatment target in cervical cancer [42]. Min et al. also used microarray analysis to identify differentially expressed genes that were induced by HPV18 E6 silencing RNA in cervical cancer [43]. Among 359 differentially expressed genes, 307 genes were up-regulated and 52 genes were downregulated in cervical cancer cells with HPV18 E6 siRNA transfection [43]. Kang and colleagues utilized DNA microarray analysis and showed that a total of 594 genes were upregulated and 651 genes were downregulated after HPV16 infection in cervical tissue [44].

The mechanisms for HPV-induced cervical cancer are complex. DNA damage response (DDR) plays a key role in cell repaired and prepares the cell for division. Viral oncoproteins combat the downstream consequences of DDR in various ways. Once the damage is unrepaired, the break points are easy for viral integration [10]. HPV infection leads to host factors including inflammatory response and oxidative stress that make initial infection such as interferon response. Interferon induces loss of episomal HPV and inhibition of E2, resulting in the selection of cells with integrated HPV genomes with higher expression of E6 and E7 [45]. Subsequently, TLR9 (Toll-like receptor 9) is downregulated and interferon response is impaired, leading to immune evasion and HPV persistent infection. Overexpression of E6/E7 promotes genetic instability and chromosomal rearrangements that enhance the risk of integration [46]. Similarly, deregulation of viral gene expression deregulates the cell cycle via p53 and Rb degradation, deregulation of oncogenes and miRNAs expression [47–50]. Without a doubt, further investigation is required to determine the detailed mechanisms of HPV-induced cervical cancer.

#### 2.1.3. DNA Methylation Microarray

DNA microarray combined with methylated DNA immunoprecipitation was conducted to analyze genome-wide methylation profiling and to identify hypermethylated biomarkers in high-grade CIN, which could be used for early detection of CIN [51,52]. Moreover, distinct DNA methylation profiles were identified by a microarray analysis between adenocarcinoma and squamous cell carcinoma of uterine cervix [53]. Among 21 genes with differential methylation pattern between two types of cervical cancers, Serine/threonine-protein kinase PAK 6 and NOGOR (NOGO receptor) could be two potential markers to be used for distinction of adenocarcinoma from squamous cancer [53]. One group performed DNA methylation microarray in normal cervical epithelium, CIN, and squamous cell carcinoma tissues. This study revealed that DNA methylation regulated microRNAs in human cervical cancer [54]. Furthermore, a total of 276 methylation genes that correlated with the prognostic status of the cervical cancer were reported based on methylation microarray analysis [55]. It has been reported that 12 candidate genes were identified by DNA methylation profiling for screening markers of detection of cervical cancer [56]. Wu et al. carried out methylated-CpG island recovery assay-based microarray analysis and found 30 genes were significantly hypermethylated in cervical cancer [57]. One group carried out methylated DNA immunoprecipitation coupled to promoter tilling arrays and identified DBC1 (deleted in bladder cancer protein 1), PDE8B (phosphodiesterase 8B), and ZNF582 (zinc finger protein 582) with frequent methylation in cervical cancer [58]. Using a CpG island microarray analysis, one group identified 6 genes, SOX1, PAX1 (paired box), LMX1A (LIM homeobox transcription factor 1, alpha), NKX6-1 (homeobox protein Nkx-6.1), WT1 (Wilms tumor-1), and ONECUT1 (one cut homeobox 1), were more frequently methylated in squamous cell carcinomas of the cervix [59]. In addition, DNA methylation analysis was performed and identified that changes in DNA methylation were related to the development of drug resistance in cervical cancer cells [60]. Liu et al. used comprehensive analysis of methylation microarray and transcriptome microarray to screen key genes and found that 561 overlapping differentially methylated genes were identified in cervical cancer. Several key genes of these identified methylated genes were associated with cervical cancer [61].

#### 2.1.4. Deep Sequencing

In addition to DNA microarray and methylation arrays, deep sequencing such as next-generation sequencing (NGS) has also been widely used to understand genetic changes in cervical cancer [62,63]. For example, one group validated 20 different HPV genotypes in 266 cervical cancer specimens using NGS approach [64]. One study described cervical cytology by deep sequencing to investigate and compare HPV metagenomes for correlation with disease states [65]. 27 different genotypes were observed in LSIL (low grade intraepithelial lesion) samples, while 17 HPV genotypes were found in HSIL (high grade intraepithelial lesion) patients. Moreover, specific HPV types E6/E7 genetic distances are associated with carcinogenic potential [65]. Another study identified pathogenesis of HPV-driven tumors, including cervical, head and neck, anal, penile and vulvar cancers using NGS and other 'omics' approaches [66]. This study shed light onto genomic HPV integration sites, disrupted genes and pathways. Common and unique genetic and epigenetic diversifications have also been determined by NGS approach in HPV-mediated cancers including cervical cancer [66].

### 2.2. Transcriptomics

Transcriptomics is the sum of all RNA transcribed for studying gene expression at RNA level, which is the link between genetic genomics and functional proteomics. Transcriptomics have been utilized to determine the molecular mechanism of carcinogenesis including cervical cancer. Specifically, microRNA array, lncRNA array, and circRNA array have been conducted to dissect the insights into mechanisms of cervical cancer.

#### 2.2.1. mRNA Microarray

One study identified the transcriptomic codes of cervical cancer using five different transcriptome datasets [67]. Hundreds of up- and down-regulated genes were reported in individual dataset. Downregulation of 113 genes and up-regulation of 199 genes were observed in cervical cancer by an analysis from all five transcriptome datasets [67]. Proteins encoded by these dysregulated core genes are involved in enzymes and modulators, hormones and signaling molecules, structural proteins, transporters, receptors, etc. The downregulated proteins include ESR1 (estrogen receptor), KAT2B (lysine acetyltransferase enzyme), FGFR2 (fibroblast growth factor receptor 2), and WNK1 (serine/threonine protein kinase). The upregulated proteins have PARP1 (poly ADP-Ribose polymerase), GSK3B (glycogen synthase kinase 3 beta), CDK1 (cyclin dependent kinase 1), and PCNA (proliferating cell nuclear antigen) [67]. Moreover, this study found that several protein deregulations were involved in metabolism pathway in cervical cancer by the integration of transcriptome data with the genome-scale metabolic network. The arachidonic acid metabolism was the key pathway, which was correlated with 15 reporter metabolites [67]. Kim et al.

performed microarray analysis of mRNA expression and found that 53 genes were differentially expressed in an early response to radiotherapy group compared with a late response group [68]. Among 53 deregulated genes, RAR-β expression is correlated to early volumetric changes to radiation therapy in cervical cancer [68].

One group used microarray analysis and described the transcriptome expression profile of irreversible senescence in HPV-positive cervical cancer cells [69]. This report defined the molecular mechanism of senescence pathway, including the induction of the RAB vesicular transport machinery and reduction of chromation regulatory molecules [69]. Wang et al. utilized three mRNA microarray datasets to investigate the important genes in cervical cancer. Furthermore, a protein-protein interaction network was utilized to further analyze these interacting genes. RhoB (Ras homolog family member B), stathmin 1 and cyclin D1 were found to be key genes in cervical cancer progression. Moreover, RhoB and stathmin 1 were identified as potential biomarkers for diagnosis and treatment of cervical cancer [70]. The microarray analysis was also used for the prediction of lymph node metastasis in cervical cancer patients. This investigation validated that RBM8A (RNA-binding protein 8A), SDHB (Succinate dehydrogenase B), SERPINB13 (serpin family B member 13), and γ-interferon could be biomarkers for prediction of lymph node metastasis in cervical cancer [71].

*2.2.2. miRNA Microarray*

MicroRNAs (miRNAs) are short noncoding RNAs with a length of approximately 22 nucleotides, which are involved in posttranscriptional regulation of gene expression [72]. MiRNAs have been identified to participate in most biological processes such as cellular differentiation and homeostasis. Furthermore, emerging evidence has revealed that miRNAs play a vital role in various diseases, including cancers. [73]. In recent years, miRNA microarray has been used to identify the expression profiles of miRNAs in cervical cancer [74–76]. For example, Li et al. reported differentially expressed miRNAs in cervical squamous cell carcinomas and adjacent non-tumor tissues using miRNA microarray including 1145 miRNAs. Seven miRNAs including miR-886-5p were differed significantly between tumor tissues and non-tumor tissues [77]. One group identified 24 miRNAs including miR-143 markedly and aberrantly expressed in cervical cancer by miRNA microarray analysis. Moreover, downregulation of miR-143 was observed in cervical cancer and overexpression of miR-143 induced apoptosis and inhibited tumor growth via targeting Bcl-2 [78]. Another group identified 15 differentially expressed miRNAs including miR-203 in cervical cancer [79]. The under-expression of miR-195-5p was determined by miRNAs microarray analysis in cervical cancer [80]. In-depth investigation showed that miR-195-5p targeted MMP-14 and suppressed cell proliferation and invasion in cervical carcinoma cells [80].

HPGD (15-hydroxyprostaglandin dehydrogenase), which suppresses cell proliferation and migration, was identified as a direct target of miR-146b-3p in cervical cancer by miRNA microarray and bioinformatics analyses [81]. One group used miRNA microarray method and found that miR-188, miR-99, miR-125b were downregulated, while miR-223 was upregulated in cervical cancer. These expression of miRNAs was associated with the short survival of cervical cancer patients [82]. In addition, miR-17-5p was confirmed to be highly expressed in cervical cancer via miRNA microarray. Furthermore, miR-17-5p was identified to promote cell proliferation and metastasis by targeting TGF-β (transforming growth factor -beta)-receptor 2 in cervical cancer [83]. In line with this, miR-374c-5p was found to be down-regulated in TGF-β1-treated cervical cancer cells by microarray analysis [84].

*2.2.3. LncRNA Microarray*

Long non-coding RNAs (lncRNAs) are defined as functional RNA molecules, which have longer than 200 nucleotides, but lncRNAs lack protein-coding capability [85]. It has been demonstrated that lncRNAs can regulate the activity of transcription factors or modulate alternations in chromatin structure [86]. An lncRNA plus mRNA microarray was conducted and revealed that 1621 lncRNA and 1345 mRNAs were differentially expressed between high-risk and low-risk squamous cervical cancer [87]. Another group reported that 5844 lncRNAs and 4436 mRNAs were differentially expressed in cervical cancer compared with normal cervical tissues [88]. Comprehensive lncRNA profiling analysis was utilized to screen differentially expressed lncRNA in cervical cancer, and observed that lncRNA NCK1-AS1 was upregulated in cervical cancer tissues [89]. Based on lncRNA microarray, lncRNA ANRIL was found to be highly expressed in cervical cancer. Downregulation of ANRIL inhibited cell proliferation, migration, and invasion in cervical cancer cells [90]. Sun et al. used lncRNA microarray and discovered that four circulating lncRNAs, including HOTAIR, PVT1, XLOC_000303, and AL592284.1, might be the potential biomarkers for prediction of cervical tumorigenesis [91]. Similarly, using transcriptome microarray analysis, lncRNA UICC was identified to be highly expressed in cervical cancer tissue [92]. Subsequent study discovered that lncRNA UICC promoted tumor growth and metastasis via regulating IL-6/STAT3 signaling pathway [92]. LncRNA ZNF667_AS1 was also identified as an independent prognostic factor of cervical cancer by microarray approach [93]. LncRNA CRNDE, which is discovered by lncRNA microarray, promoted cell growth and metastasis in cervical cancer cells [94]. Similarly, lncRNA SNHG1 was identified using lncRNA microarray in cervical cancer, and SNHG1 promoted cell proliferation, migration and invasion in cervical cancer cells [95].

LncRNA microarray was also used to screen the differentially expression profiles of lncRNAs in early stage cervical cancer patients and found a total of 2574 upregulated lncRNAs and 3270 downregulate lncRNAs. Among these lncRNAs, overexpression of lncRNA RP11-396F22.1 was correlated with poor prognosis in early stage cervical cancer patients [96]. LncRNA microarray and lncRNA-mRNA co-expression analysis were utilized to determine the expression of lncRNA in cervical cancer cells. This study demonstrated that 4750 lncRNAs were differentially expressed in HPV-16 positive cells compared with HPV negative cells. Among these deregulated lncRNAs, 2127 lncRNAs were upregulated, while 2623 lncRNAs were down-regulated. There were 5026 lncRNAs that were differentially expressed in HPV-18 positive cells compared to HPV negative cells, including 2218 upregulated and 2808 downregulated lncRNAs [97]. One study by Sun et al. also identified differentially expressed lncRNAs and mRNAs in cervical cancer compared with peritumoral tissues by transcriptome microarray analysis. In fact, 708 lncRNAs were increased and 836 lncRNAs were decreased in cervical cancer tissues. Moreover, 1288 mRNA levels were increased and 901 mRNAs were decreased in cervical cancer. Strikingly, lncRNA EBIC interacted with EZH2 and inhibited E-cadherin and enhanced tumor cell invasion in cervical cancer [98]. Zhu et al. also reported that 3356 lncRNAs have significantly different expression pattern in cervical cancer tissues compared with adjacent normal tissues, including 1857 upregulated lncRNAs [99]. Two lncRNAs (lncRNA01101 and lnc00277) were selected and validated as novel factors from hundreds of lncRNAs that were identified by microarray analysis in HPV-induced cervical cancer [100].

*2.2.4. circRNA Microarray*

Circular RNA (circRNA), a special class of non-coding RNA, forms a covalently closed continuous loop in which the 3′ and 5′ ends have been joined together [101]. This feature confers numerous properties to circular RNAs, such as resistance to RNA exonuclease activity, stable expression and uneasy to degrade. The circRNA plays the important role in modulation of gene expressions in human cancers, by sequestration or sponge other gene expression, especially miRNA. [102]. Recently, it was reported that HPV-16 E7 oncoprotein altered the expression profiles of circular RNAs by high-throughput microarray technology in cervical cancer cells [103]. In total, 526 dysregulated circRNAs were changed by HPV E7 expression, including 352 upregulated and 174 downregulated circRNAs [103]. Emerging evidence has suggested that circular RNAs (circRNAs) are critical regulators in the cancer

development and progression. It has been documented that microarray is an efficient tool for circRNA profiling [104]. One study showed that about 80,000 circRNAs were detected in cervical cancer samples and matched normal tissues, and about 25,000 of them were differently expressed [104]. Abnormally expressed circRNA in cervical cancer cells has been detected by human circRNA microarray screening. Among these circRNAs, circRNA-000284 was upregulated in cervical cancer cells and promoted cell proliferation and invasion via sponging miR-506 [105]. CircRNA expression profiles defined that 45 expressed circRNAs have 4 fold changes in cervical cancer tissues compared with adjacent normal tissue. Among these dysregulated circRNAs, circ-0018289 was up-regulated and promoted the tumorigenesis in cervical cancer [106].

#### 2.2.5. High-throughput RNA Sequencing

Recently, many groups use high-throughput RNA sequencing to explore the expression profiles of mRNAs, miRNAs, circRNAs, and lncRNAs in cervical cancer [107–109]. For example, using RNA sequencing, transcriptome profiling of the cancer and adjacent normal tissues from cervical cancer patients has been analyzed [109]. There are 347 differentially expressed genes (DEGs), including 104 upregulated and 148 downregulated DEGs in cervical cancer [109]. Similarly, 40 upregulated genes and 3 downregulated genes have been discovered in HSIL using RNA-seq [110]. One study identified 304 mRNAs, 28 miRNAs, 99 circRNAs, and 19 lncRNAs that were differentially expressed in cervical cancer patients [107]. Another study has validated significantly expression variation of six miRNAs between human cervical cancer lines and normal cervical cells through a direct sequencing method [108]. Furthermore, it displayed that miR-143 was decreased expression while miR-21 was increased in cancer samples, indicating that these miRNAs could be tumor markers [108]. Moreover, RNA-Seq analysis was carried out to characterize HPV integration, viral gene expression and E6E7 alternative transcripts in cervical cancer [111]. RNA sequencing is a powerful tool to explore the expression profiles of RNAs in cervical cancer.

### 2.3. Proteomics

With the gradual completion of the human genome project, proteomics emerges which requires reliable and high-throughput technologies such as mass spectrometry and tissue microarray [112]. Genetic changes will eventually lead to changes in the protein expressed. Proteins synthesized in the cervix are modified by phosphorylation, glycosylation and acetylation, making their composition more complex than genes. Therefore, it is possible to describe and predict complex life activities by using proteomic techniques to study the changes of various proteins in life activities. Proteomics has been widely employed in cervical oncogenesis. Gene-encoded proteomics compares the changes of protein profiles in cervical cancer with normal cervix by establishing a complete protein library to find out the differential proteins before and after the occurrence of cervical cancer. Proteomics provides a new clue for early search for new biomarker proteins and elucidates the pathogenesis of cervical cancer.

Lee et al. used proteomics and genomics to identify protein profiling and modulators regulated by the E7 oncogene in the cervical cancer cells. Protein disulfide isomerase A3, integrase interactor 1 protein, glutathione S-transferase P, and vav proto-oncogene were decreased, while heat shock 60 kDa protein 1, Ku70 binding protein, alpha enolase were increased in cervical cancer after E7 induction [113]. The result from a genomic method demonstrated that IL-12R beta 1, cytochrome *c*, tumor necrosis factor receptor II were increased due to E7 overexpression. Therefore, E7 could evade immune surveillance via regulation of multiple molecules in cervical cancer [113]. Using proteomic microarray and qRT-PCR approaches, it has been found that increased cycling cell numbers and stem cell associated proteins, including chorionic gonadotropin, TP63 (Tumor protein p63), SOX2 (SRY-box 2), could be potential biomarkers for high grade HPV positive CIN3 [114]. Zhu et al. used two-dimensional electrophoresis and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry to identify differentially expressed proteins in squamous cervical cancer [115]. This study identified 55 dysregulated proteins, including 24 upregulation and 31 downregulation proteins in cervical cancer [115]. The overexpression of S100A9 was further confirmed by immunoblotting and immunohistochemical approaches in cervical cancer [115,116].

A protein microarray, also termed a protein chip, is created by large number of proteins arrayed on a surface enabling the simultaneous study of protein functions and interactions in array technologies [117]. The protein microarray platform is particularly suited for unbiased global profiling [118]. PTEN (phosphatase and tensin homolog deleted on chromosome ten) expression was evaluated in cervical cancer by tissue microarray [119]. This study identified that majority of cervical adenocarcinoma patients have PTEN expression, which was correlated with histologic subtypes of adenocarcinoma [119]. Similarly, RB pathway was also assessed in 265 paraffin-embedded samples of cervical intraepithelial neoplasia by immunohistochemistry applied to a tissue microarray [120]. This group reports that low expression of p16 (INK4a) is associated with prognostic significance to predict recurrence, suggesting that p16 level could be used for stratifying patients for different treatment strategy [120]. Additionally, the expression of senescence and apoptosis markers was determined using immunohistochemical staining in tissue microarray in cervical cancer. The results demonstrated that p15 (INK4b), p16 (INK4a) and p14 (ARF) levels were increased in cervical carcinoma, indicating that senescence and apoptotic pathways could be involved in cervical tumorigenesis [121]. Using a tissue microarray and IHC approach, one group revealed that up-regulation and nuclear localization of VHR (VH1-related) were observed due to its post-translational stabilization in cervical cancer cell lines, demonstrating that VHR might be a potential novel marker and therapeutic target for cervical carcinoma [122]. Using tissue microarray, MMP-2 (matrix metalloproteinase 2) was identified to be highly expressed in cervical cancer tissues and correlated with lymph node metastasis and parametrium invasion. Interestingly, MMP-2 level is not associated with recurrence and survival in patients with cervical cancer [123]. Wan et al. utilized oligonucleotide microarray and tissue microarray to reveal that SIX1 and GDF15 could be two potential biomarkers of cervical cancer progression [124]. Similarly, high level of human nm23-H1 (nonmetastatic clone 23 type 1) was reported to be associated with poor differentiation and worse overall survival using tissue microarray approach in cervical cancer [125]. MEKK3 (mitogen-activated protein kinase/extracellular signal-regulated kinase kinase kinase 3) and survivin expressions were associated with clinical stage, infiltration depth, and lymph node metastasis in cervical cancer using tissue microarray and immunochemistry approaches [126]. In addition, KLF5 (Krueppel-like factor 5) was confirmed as a potential molecular marker in cervical cancer by a tissue microarray [127]. Expression of FGFR (fibroblast growth factor receptor) family members has been validated to be correlated with prognosis in early stage cervical cancer using a tissue microarray [128]. Recently, high expression of SEL1L (sel-1 homolog 1), Notch3 (Notch homolog 3) and SOCS3 (suppressor of cytokine signaling 3) was identified by tissue microarray in cervical cancer patients [129]. The study from Chen and colleagues confirmed that FOXM1 (forkhead box M1) and the Hh signaling pathway participate in cervical cancer by tissue microarray analysis. They also indicated that FOXM1 may be a downstream target gene of the Hh signaling pathway in cervical cancer, which provides a potential novel diagnostic and therapeutic target for cervical cancer [130]. In addition to proteomics to identify total protein expression changes, protein tyrosine phosphorylation changes have been measured in cervical cancer using phosphorproteomics [131]. This study revealed that Annexin A1 as well as DNA-PKcs (DNA-dependent protein kinase, catalytic subunit) could have synergistic effects with HPV infection [131]. Similarly, nine phosphorylation sites of Mcl-1 in response to microtubule targeting agents were identified using two-dimensional gel electrophoresis and

phosphoproteomics, implying that Mcl-1 phosphorylation is required to further dissect its function and role in cervical cancer [132].

### 2.4. Metabonomics

Metabonomics is to use modern analytical methods to study the metabolic products of endogenous small molecular substances such as plasma, urine, tissue homogenate and cells to reveal the metabolic essence of life activities [133]. It has been known that cancer development and progression often have accompanied with metabolic changes. Metabolomics has been used to measure cancer metabolism and to identify altered metabolites and pathways in tumor initiation and progression [133]. Metabolomics is high efficiency because it does not need full genome sequencing and a large number of expressed sequence tags (EST) and are far less than the number of genes and proteins [134]. Over the past decade, cancer metabolic research is revived, and particularly two outstanding characteristics have gotten the center of attention: (1) Warburg effect: an increased glucose uptake rate and secretion of lactate even in the presence of oxygen and (2) glutamine addiction: a high glutamine uptake rate is necessary for cell growth [135]. Understanding cancer metabolism is important, which could become a focus of chemotherapeutics [136]. A recent research shows the changes of oncoproteins E6 and E7 in glycosylation, and lymph invasion has related to the expression levels of some glycogens in cervical carcinoma. 9 upregulated glycogens and 7 downregulated glycogens in HeLa shE6/E7 cells have been reported using the microarray analysis [137]. One study has shown that 117 genes were differentially expressed, in which most genes were involved in regulation of catalytic activity using transcriptomics analysis between cervical cancer patients and normal controls [138].

Metabolomics were also applied to cervical cancer using 1H nuclear magnetic resonance ($^1$H NMR). Ye et al. detected metabolomics profiles of serum samples from patients with cervical cancer, CIN, and chronic cervicitis, respectively. They found that the main metabolites, including formate, tyrosine, β-glucose, inositol, glucine, carnitine, glutamine, acetate, alanine, valine, isoleucine, and VLDL (very low density lipoprotein), could be contributed to these discriminations [139]. This study indicates that the systemic metabolic response might validate the potential biomarkers for cervical cancer. Chai et al. used 1H NMR combined with chemometric analysis to generate metabolic profile data in fecal samples of cervical cancer patients with radiation-induced acute intestinal symptoms (RIAIS) [140]. The different metabolic profiles were developed not only between the pre- and post-radiotherapy RIAIS patients, but also between RIAIS patients and controls, suggesting that this profile could be useful for RIAIS diagnosis or therapeutic monitoring [140]. Yin et al. used UPLC-MS (ultra-performance liquid chromatographic-mass spectrometry) to detect the molecular metabolite in plasma of squamous cervical cancer, and identified two metabolites, phosphatidylcholine and lysophosphatidylcholine, as novel potential biomarkers for cervical cancer [141]. Moreover, a metabolomics approach has been carried out to predict the response to neoadjuvant chemotherapy in cervical cancer patients [142]. L-valine and L-tryptophan have been validated as the potential biomarkers for patient response to chemotherapy [142]. Furthermore, metabolic signatures in plasma were assessed by high-performance liquid chromatography in CIN and cervical squamous cell carcinoma. Compared with healthy controls, lower levels of plasma amino acids were observed in CIN and cervical cancers. Arginine and threonine levels were upregulated in plasma of CIN patients, while there levels were downregulated in cervical cancer. Moreover, the plasma levels of a larger group of amino acids were gradually decreased from CIN to invasive cervical cancer. This study suggest that plasma-free amino acid profiles could be useful for helping cancer diagnoses in the early stage using blood samples and metabolomic analysis [143].

### 2.5. Other Systems Biology Approaches

Recently, commensal bacteria were revealed to be a major factor in both healthy and disease pathogenesis through human microbiome research. It is expanded beyond the gut to other organ systems, especially as vaginal microbiome [144]. Many studies have proved the association between the vaginal microbiome and CIN [145,146]. Previous studies have shown that a great deal of vaginal flora such as mycoplasma genitalium, aerobic lactobacilli, *Staphylococcus epidermidis*, enterococci, *Escherichia coli*, and bacteroides species in cervical cancer patients are diverse compared with that in healthy controls [147,148]. In a word, microbiomes rich in *L. crispatus* were connected with healthy patients while lactobacillus inners associated with higher grades of CIN in HPV-positive patients and cervical cancer [146,149]. The epigenomics emerged recently is the research of epigenetic changes at the genomic level. The essence is to alter the modification of the genome involved in DNA methylation, histone modifications without affecting the DNA sequence, thereby affecting individual development, and this change also can be inherited [150]. DNA methylation changes and histone modifications are being studied in cervical cancers of epigenetic regulation of gene expression. As mentioned above, DNA methylation in the promoter and upstream CpG islands has been contributed to cervical carcinogenesis in several tumor suppressor genes including cell cycle, apoptosis, DNA repair, cell differentiation, transcription, and signaling pathway [150]. Phosphorylation and acetylation of histone H3 were reported significant association with cervical cancer progression [151].

## 3. Conclusion and Perspective

Systems biology approaches have been utilized to explore the molecular mechanism of cervical cancer development and progression. DNA microarray and tissue microarray have been performed to determine the biomarkers of prognosis and treatment outcome in cervical cancer. Transcriptomics is the link between genetic genomics and functional proteomics. Specifically, microRNA array, lncRNA array, and circRNA array have been conducted to dissect the insights into mechanism of cervical carcinoma. It is necessary to mention that the effective combination of genome, transcriptome, proteome and metabolome could be a better approach to explore the molecular mechanism and to identify biomarkers for cervical cancer prognosis. The common pathways or targets could be identified in cervical cancer by genome, transcriptome, proteome approaches. For example, p16 level was found to be downregulated by DNA microarray [35] and tissue microarray [121]. However, inconsistent results were also found by different approaches. For instance, p53 was observed to be decreased by DNA microarray [37], whereas its expression was increased by tissue microarray [121]. These controversial findings suggest that identified targets by each approach must be further validated by other multiple methods.

We think that new systems biology approaches will be discovered to define the molecular basis of cervical tumorigenesis, and to impact on translation medicine of cervical cancer in the near future. Moreover, in the screening of drug therapy for cervical cancer, such as single drug active site or multi-link, multi-target integration and regulation of drug combination, systems biology approach will become an important way. In addition, appropriate statistical analyses would be of great help for systems biology methods. Due to too comprehensive data from system biology approach, it is difficult to select the key pathways and important targets involved in cervical cancer development, progression, and treatment. In a word, using systems biology technology will largely help us to study the pathogenesis of cervical cancer and seek diagnostic accuracy, predictive performance and effective treatment for current basic and clinical study. However, it has a long way for better personalized therapy of human cancer patients by system biology technologies.

## Conflicts of Interest

The authors declare that they have no competing interests.

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