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REVIEWS

Modern Approaches to Prevention and Control of Cancer of the Uterine Cervix in Women

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Abstract | Cancer of uterine cervix is a major reproductive public health problem in developing countries. Infection of human papillomavirus (HPV) mainly with high-risk HPV Type 16 is the most crucial event in malignant transformation and the leading cause of cervical cancer in women. Early cancer detection using newer diagnostic modalities should improve the clinical outcome by detecting the disease at an early stage and thus minimizing the morbidity and improving survival. Since persistence of oncogenic HPV infection is important for tumorigenic transformation, analysis of their physical status has prognostic significance. Although two successful prophylactic HPV vaccines (Gardasil and Cervarix) are available for primary prevention, the extremely valuable effects of cytological Pap smear screening, VIA and HPV DNA testing cannot be ruled out. This paper reviews the use of prognostic biomarkers, different prevention strategies and newer therapeutic approaches against cervical cancer.

Keywords: *Cervical cancer, HPV, HPV vaccine, Screening, Micro-RNA, Cancer stem cell*

1 Introduction

Cervical cancer is third most common cancer among Indian women though breast is the leading cancer site globally. In India, cervical cancer has increased from 0.11 million in 2000 to 0.16 million in 2010.[1,2] The proportion ranged from 15% to 55% of female cancers from different parts of the country. Over 80% of the cervical cancer present are at a fairly advanced stage, and annually around 80,000 deaths are reported in India.[1] According to global cancer statistics, cervical cancer is now the third most commonly diagnosed cancer and the fourth leading cause of cancer death in women worldwide, accounting for 9% (529,800) of the total new cancer cases and 8% (275,100) of the total cancer deaths among females in 2008. More than 85% of these cases occur in developing countries. India, the second most populous country in the world, accounts for 27% (77,100) of the total cervical cancer deaths.[3] The disproportionately high burden of cervical cancer in developing countries and elsewhere in medically under-served populations is largely due to lack of screening that allows detection of pre-cancerous and early stage cervical cancer.[3,4]

It is now well recognized that cervical carcinogenesis occur in a stepwise fashion. The transition of normal epithelium to pre-neoplastic lesions, and to invasive carcinoma occurs sequentially and progresses through well recognized stages, and takes approximately 10–20 years to develop an overt malignancy. In India, cervical cancer most often develops around the age of 45 years and peaks at 55 years of age. Of the several risk factors that have been implicated in the causation of cervical cancer, persistent infection of human Papillomavirus (HPV), a double-stranded DNA virus, is considered a principal sexually transmitted casual agent in the development of cervical cancer. To date, more than 140 human and animal papillomavirus genotypes have been characterized and sequenced. Of the approximately 30 HPVs that infect the anogenital tract, 15 HPV types, classified as 'high-risk' types (HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82), are associated with high grade lesions and invasive cervical cancer.[5] Molecular and clinicoepidemiological studies have demonstrated that HPV types 16 and 18 are the two most common among these. On the other hand, 11 different HPV types,

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classified as 'low-risk' HPV types (HPV types 6, 11, 40, 42, 43, 44, 54, 61, 70, 81 and CP6108), are mainly associated with genital warts and benign cervical lesions. Among these, HPV6 and HPV11 cause approximately 90% of the genital warts. In addition to HPVs, cofactors such as parity, early age of marriage, genital hygiene, promiscuity, use of oral contraceptives, smoking, immune suppression, infection with other sexually transmitted agents and poor nutrition have been associated with the development of cervical cancer.[6,7,8]

An analysis of pooled data revealed that at least eight HPV types 16, 18, 31, 35, 45, 52 and 58 account for almost 95% of cervical cancers.[4] In India, the prevalence of HPV in cervical intraepithelial lesions and cancer is > 80% and the high risk HPV type 16/18 present in about > 90% of cervical cancer cases. A population-based, cross-sectional survey in married women aged 16–59 years was conducted in rural Dindigul district, Tamilnadu.[8] The predominant type was HPV 16, followed by HPV 56, HPV 31, HPV 33 and HPV 18. India not only contains large geographical diversity but also has contrasting cultural variations and different religions that have been shown to influence the sexual behavior of women and their male partners, leading to differential acquisition of new HPVs.[9] In a national HPV mapping study in India using Southern blotting, the prevalence of HPV16 was found to be highest in Chennai (88%), and lowest in Jammu and Kashmir (14.2%).[10,11] Most interestingly and in contrast to most of the Western population, the prevalence of HPV infection in young adolescents is much lower (3%) and the peak is more blunt as well, as is observed in the latter half of the third decade of life in Indian women.[12,13] Considering the scenario, there appears to be an urgent need for introduction of effective therapeutics as well as prophylactic vaccines against high risk HPVs is essential. A major thrust in the National Cancer Control Program has been to detect cancer at an early stage. Targeted interventions can lead to a decrease in the projected increase in cancer burden through effective primary prevention strategies, alongside implementation of vaccination, early detection, and effective treatment programmes.

2 Prevention of Cervical Cancer through Screening

The current relationship between cervical cancer and viral infection provides a unique platform for detection of HPV infection and associated cellular changes using visual, cellular and molecular techniques. However, most genital HPV infections are asymptomatic and unapparent in the beginning, and molecular tests are the most preferred and reliable methods for early detection. Conventionally, early detection of cervical cancer lesions are achieved by conventional cytology using Papanicolaou smear test (or Pap test) as a primary screening tool. However, several studies have repeatedly shown that Pap smear test itself has several limitations such as high false negative rates as high as 20–30% coupled with low sensitivity (about 45%), subjective interpretation, weak quality control, inability to identify latent HPV infection and low predictive value. Despite poor sensitivity and specificity, this test has shown remarkable results in USA and other developed countries where it has been incorporated in routine screening of women aged 35 years or older. Due to lack of experienced cyto-screeners and other trained manpower and infrastructure, use of cytologic Pap test as well as development of cytology-based screening program in India are still far from adequate. Therefore, a number of alternative methods have been employed for the detection of cervical cancer precursors, specifically the visual inspection methods (VIA) that are suitable for low resource settings by paramedical staff, and more recently the HPV DNA testing which provides unequivocal results with respect to ASCUS and AGUS. HPV DNA testing is important since majority of HPV infections are transient and asymptomatic and are undetectable by cytology or VIA.[14] Once precancerous lesions are identified they can be subjected to cryotherapy, loop electrosurgical excision procedure or cold knife conization having effectiveness of upto 95% depending upon the degree of lesion. However, these methods not being HPV-specific, the patients should be triaged and periodically tested for recurrence. Cervical screening for women is necessary because there are often no signs and symptoms of cervical pre-cancers. The establishment of a prevention program is essential considering both screening and vaccination. But most women in India do not have access to effective screening programmes. It has been estimated that in India, even with a major effort to expand cytology services, it will not be possible to screen even one-fourth of the population once in their lifetime in the near future. The focus on detection and prevention of cervical cancer must be emphasized in a highly populated country like India to prevent its extensive spread. Although two effective prophylactic vaccines are available and since they provide protection only to vaccine specific HPVs (HPV 16, 18, 6 and 11), screening has to continue to avoid development of cervical cancer due to infection of other high risk HPV types.

HPV DNA testing—an alternative screening method: Since HPV cannot be cultured in vitro system and unlike other infectious viruses for which serological tests are most frequently used, HPV testing relies mainly on DNA/RNA-based molecular diagnostic methods that detect the viral genome in cervical specimens.[7] Owing to multiple HPV types with varied oncogenic potential, HPV tests are designed to determine if a patient is infected with one or more high-risk HPV types. The need for sensitive HPV detection arises from the fact that detecting HPV infection at an early stage could provide a means of triaging the patient over a period of time to distinguish a transient infection from the persistent one that could be more detrimental to the patient. Therefore, through large scale population screening using high through-put molecular diagnostic tests, it is possible to identify the high risk group of women that are predisposed for developing cervical cancer. Several nucleic acid-based detection techniques presently available for the detection of HPV infection are given in Table 1.

3 Predictive and Prognostic Biomarkers

HPV DNA testing as the primary biomarker for early detection of pre-cancer lesions and triaging: Direct detection of HPV DNA in cervical specimens may offer an alternative or complementary to population-based cytological screening. It has been reported that HPV test results are more sensitive than Pap smears in detecting high-grade lesions in older women.[12,14,25,26] Studies also support the potential utility of HPV testing for effective triaging of Pap smears of atypical squamous cells of undetermined significance (ASCUS) and atypical glandular cells of undetermined significance (AGUS), and therefore have a potential role in primary screening of populations in which Pap smears have been inconclusive. DNA probes of high-risk HPV types in different formats have been fully validated as primary screening tests, secondary triage tests, and as a prognostic marker following treatment of high grade squamous intraepithelial lesions (HSIL). They consistently showed significant superiority over the conventional Pap smears.

HPV E6 and E7 protein detection in the severe dysplastic and invasive carcinoma cases: Since two early genes E6 and E7 are the main viral transforming genes that are invariably retained in almost all cervical cancers, detection of these two viral oncoproteins can serve as important biomarkers of severe dysplastic and invasive cervical cancers and progression of the disease. HPV E6 and E7 oncogenes play an essential role in HPV-induced carcinogenesis by interfering with two essential tumor suppressor genes p53 and pRb that regulate normal cellular proliferation and serve as important and better biomarkers than HPV DNA for early detection of cervical cancer. Studies show that detection of E6/E7 mRNA expression could predict the risk of cervical cancer.[23] Recently, commercially available mRNA-based assays (e.g., NucliSENSEasyQ HPV Test and APTIMA HPV mRNA Assay) are also being used. ArborVita Corporation has developed a rapid diagnostic test, "AV Avantage HPV E6 test" in collaboration with PATH (Program of Appropriate Technology in Health) with FDA approval, and is expected to be launched in 2013. AV Avantage HPV E6 test uses a high-affinity monoclonal antibody for the detection of E6 oncoprotein from high risk HPV 16, 18, and 45 responsible for approximately 90% of cervical cancers.[14,27]

HPV E5 protein detection in precancerous lesions: The E5 protein is expressed in precancerous stages of cervical epithelium during HPV

Table 1: An overview of nucleic acid-based detection techniques used for the detection of HPV infection.

Category	Target	Test methods	References
Direct Probe Method		Southern blot hybridization, in situ hybridization (ISH), Filter in situ hybridization (FISH) and dot bot	15, 16
Signal Amplification	DNA	Digene's Hybrid Capture 2 (HC-II), Rapid HPV test, Cervista HR-HPV test, Ventana Inform HPV and Dako Gen point (Dako)	17, 18
Target Amplification	A	Consensus PCR, multiplex PCR, PCR-ELISA, GP5+/6+-PCR-EIA assay and Real Time PCRs. Cobas 4800 HPV test, SPF10-LiPa, MGP PCR-Luminex, PGMY RLB, Linear array, DNA chip, Digene HPV genotyping RH test, Digene HPV genotyping LQ test, Amplicor and PapilloCheck	5, 19, 20, 21
	mRNA	HPV E6/E7 mRNA detection by RT-PCR, PreTect HPV, Proofer Assay, NucliSENSEasyQ HPV v1 Assay and Aptima HPV assay	22, 23, 24

infection. Since precancerous lesions usually contain fewer cells than the invasive malignancies, it can be speculated that early immunological intervention might offer a chance to eradicate tumors more efficiently at this premalignant stage. Furthermore, cells in more advanced stages are found to have very low expression levels of MHC class I and II mRNA, which may consequently hamper the presentation of tumor antigen and lead to decreased immunosurveillance. Some reports indicate that lymphocyte proliferation in response to HPV16 E5 is inversely proportional to the severity of the cervical squamous intraepithelial neoplastic lesions (CSILs).[28] Hence, presence of the E5 protein in precancerous lesions can be used as a prognostic marker.[29]

Viral load and Integration: There exists a close link between HPV viral copy number and integration of viral genome into the host cell and is considered as a risk factor for the progression of pre-cancer to invasive cancer.[30,31] The significantly higher HPV load detected in women with high-grade cervical dysplasia, as well as the dramatic difference in the load after surgical removal of the lesion, suggest that HPV load is a possible prognostic marker of high-grade squamous intraepithelial lesion. Integration of the viral DNA into host cell genome is yet another biomarker as persistent HPV infection is essential for the integration of viral DNA into the host cell genome, leading to tumorigenic transformation of cervical epithelium.

A number of molecular markers have been found to show an early sign of alteration at the onset of the disease which may be useful in predicting the disease course at an early stage.

p53, the guardian of the genome has been found to be deregulated with the progression of cervical lesions, suggesting that p53 abnormality is an early event in cervical carcinogenesis. The E6 protein of oncogenic HPV types has been shown to complex with p53 and target it for rapid degradation. As a consequence, p53's growth-arrest and apoptosis-inducing activities are abrogated. This suggests the potential importance of the E6—p53 interaction for therapeutic intervention.[41]

p16, the cyclinD/cdk inhibitor is overexpressed as the lesions proceed to a more aggressive one. This tumor suppressor p16/INK4A plays an important role in regulating the cell cycle and is overexpressed in the presence of HPV E7 oncoprotein. Several studies reported p16/INK4A as a useful diagnostic marker for squamous and glandular epithelial dysplasia in the uterine cervix[32,33] and a valuable surrogate marker for high risk and malignant cervical lesions in the presence of HPV.[34] Furthermore, expression of p16/INK4A appears to correlate with the degree of cervical neoplasia.[35,36] A recent study showed that a p16/INK4A immunocytochemical assay has better specificity than HPV testing to predict underlying high-grade dysplastic lesions.[37] Currently, clinical trials are underway to assess the diagnostic and prognostic value of p16/INK4A expression in atypical glandular cells and low-grade squamous intraepithelial lesions of the cervix.

c-fos protein specifically shows exclusive high expression with increasing severity of lesion and in cancer.[12] The transcription factor AP-1, which is composed of heterodimer of members of the c-Jun and c-Fos families, plays an indispensable role in HPV oncogene expression and regulates various other cellular processes such as enhanced proliferation, apoptosis and tumor metastasis. In particular, among the various members of AP-1, c-fos acts as a tumor promoter and its upregulation causes cellular transformation. In addition, when c-fos binds to c-jun, it increases the gene expression of cyclinD1 and contributes to the potentiation of malignancy. Therefore c-fos regulation plays a fundamental role in HPV-induced tumor development.[38,39]

Fra-1 is over expressed in normal cervical tissue and its expression is diminished as the lesion progresses from pre-cancer to cancer. Thus, in cervical cancer it acts like a tumor suppressor gene.[39]

p50-RelA subunit of NF-kB shows enhanced expression in high grade cervical lesions and changes in relation to disease progression.[40] Therefore, the detection of these subunits of NF-kB in cervical cancer tissues serves as a useful prognostic marker. Also these subunits can be potentially used as specific targets for developing therapeutics for human cervical cancer.

NOTCH 1 family of proteins is found to be highly expressed from lesions CIN III onwards. Activated Notch1 inhibits p53-induced apoptosis and sustains transformation by HPV Type 16 E6 and E7 oncogenes through a PI3K-PKB/Akt-dependent pathway. Thus, activation of Notch signaling may serve as an additional mechanism to inhibit wild-type p53 function in papillomavirus-associated neoplasia.[41,42]

Rb protein has been found to be deregulated in poorly differentiated carcinoma, suggesting its important role in differentiation. pRB is a negative regulator of the cell cycle that normally prevents S-phase entry by associating with the E2F family of transcription factors. E7 binding to pRB releases E2F, irrespective of the presence of external growth factors, leading to the expression of proteins necessary for DNA replication.[43]

Telomerase activation is a relatively early event in cervical carcinogenesis and mostly correlated with the grade of cervical lesion, HR-HPV status (HPV 16 and 18 subtypes) and clinical staging. Upregulated hTR and hTERT subunits of telomerase have also been observed in cervical cancers.[44] A study by Kailash et al. suggested that telomerase activation is a relatively early-stage event in cervical carcinogenesis, and this activation is associated with the initiation and progression of cervical lesions. However, detection of telomerase activity may serve as a biomarker for reliable diagnosis and prognosis of cervical neoplasia.[43]

Ki-67 is a nuclear protein that is expressed during all active phases of the cell cycle, and its expression is used to determine the cell proliferation status. In cervical intraepithelial neoplasia (CIN), Ki-67 expression is increased in the upper layers of cervical epithelium compared to normal cervices.[46] Several studies have also suggested that Ki-67 can be used as an independent prognostic marker to identify women with high risk for progression and/or recurrence of cervical squamous precancerous lesions.[47]

E.Cadherin, Cadherins are glycoproteins of 120 to 130 kDa that are involved in cell adhesion and are considered as important biomarkers for tumor development.[48] The squamous cells of cervical epithelium are strongly attached to each other and to the basement membrane through a large number of adhesion molecules. Thus, E-cadherin is one of the key molecules of adhesion. The decrease or loss of expression of these molecules can be correlated with aggressive behavior and progression of cancer. The decrease in the expression of E-cadherin seems to be a useful parameter in evaluating the potential for malignancy of cervical cancer.[49]

3.1 *Potential serum markers*

SCCA (Squamous cell carcinoma antigen) for cervical cancer, the discovery of useful serum biomarkers for its early detection has been a priority now-a-days. Such tumor markers are the molecules gaining importance owing to the presence of tumor, which can appear in the surrounding tissue and then within the blood and excretions. Diagnostic serum markers for cervical cancer in clinical use are SCC antigen.[50]

Cell adhesion matrixproteins CD44 and its variant forms are integral membrane proteins that have been implicated in tumorigenesis. They act as both, in a lymphocyte homing mechanism and cell adhesion molecule as well as being involved with tumor growth, spread, and invasion. CD44 is generally used as an epithelial cancer stem cell marker and thus provides novel approach to the diagnosis and treatment of cancer.[51]

4 Prophylactic HPV Vaccination

Due to a substantial lag period of 10–15 years between the time of HPV infection and development of invasive cervical cancer, and a high possibility of reversal of precancerous lesions to normal at an early stage, cervical cancer is an easily curable disease which can be controlled by both primary and secondary prevention. This can be achieved by either preventing the entry of HPV to block the establishment of viral infection by vaccination, or detecting the precursor lesions at an early stage through screening. Latter facilitates identification of early lesions that can be cured by providing localized treatment. Recently, however, two clinically approved prophylactic vaccines against HPV have become commercially available, providing the first really effective means of preventing infection with HPV, ultimately leading to the prevention of development of cervical cancer.

One of the most effective and well accepted primary prevention methods for a disease of infectious origin is through vaccination. Clinical goals of HPV vaccination are prevention of cervical carcinoma, prevention of cervical dysplasia, symptomatic anogenital disease and genital warts as well as prevention of other HPV-related cancers like head & neck carcinomas. Two extremely immunogenic virus-like particles (VLPs)-based vaccines, Gardasil® and Cervarix®, have been developed and approved clinically worldwide that generate robust immune response following administration of 3 doses (0, 1 or 2 and 6 months) in over 99% of recipients preventing new infection with these viruses.[52] Both, Gardasil—a tetravalent (against HPV16, 18, 6, 11) vaccine developed by Merck, USA as well as Cervarix—a bivalent (HPV16,18) vaccine developed by Glaxo-SmithKline (GSK), Belgium are recommended for vaccinating young adolescent girls, i.e. girls aged 9–13 years at or prior to the onset of puberty.[53] Concerns regarding vaccine safety arose shortly after vaccine distribution in larger populations of children and adolescent girls. The documented adverse events after vaccination included mostly local site reaction, dizziness, nausea, headache, and hypersensitivity reactions including urticaria. Overall reporting rates were as expected for other vaccines, but disproportional reporting of syncope and venous thromboembolic events were also noted. Even though the trials excluded pregnant women, many women became pregnant during the evaluation phase; based on these data, both vaccines show good safety profiles.

5 Upcoming HPV Vaccines

The current quadrivalent and bivalent L1 VLP based vaccines provide only limited cross-protection to development of persistent infection and CIN 2–3/AIS caused by non-vaccine HPV types. Thus, a multivalent vaccine against a multitude of HPVs will be a major breakthrough in providing near-complete prevention of HPV-related diseases, and indeed, efforts to develop a nine-Type L1 VLP combination vaccine are under research.[54] Pre-clinical and human volunteer studies have also suggested that immunization against the minor capsid protein, L2 with the candidate prophylactic/therapeutic vaccine HPV16-L2E6E7 might work as a broad spectrum against different genotypes of HPVs.[55] Development of low-cost vaccines using plant species such as tobacco, potatoes and tomatoes for the production of VLPs is also underway.[56] Although the available prophylactic vaccines have no therapeutic effects, it will take decades before their use will markedly reduce the frequency of HPV-associated cancers. In contrast, HPV-specific therapeutic vaccines would have an urgent impact on cervical cancer reduction.

Therapeutic vaccines incorporating E6E7 proteins such as the HPV16 E6E7 ISCOMATRIX vaccine are being investigated for treatment of HPV-related anal intraepithelial neoplasia in HIV-infected men. In addition, various strategies are being evaluated to generate a combined therapeutic and prophylactic vaccine called chimeric vaccines, and this vaccine will prove more useful in Indian context. A VLP-based vaccine consisting of an L1/E7 chimera has also been tested in patients with HPV16-positive precursor lesions. It was safe, but only showed moderate therapeutic efficacy.

Development of therapeutic potential to these vaccines is also being carried out for the last two decades which could simultaneously eliminate HPV infections and cure associated lesions. The main goal of future vaccine is to develop vaccines that will be more suitable to resource limited countries, that is, to reduce the cost of production, to have a longer shelf-life, single dose delivery, long lasting immunity, no requirement for refrigeration, and incorporation of other oncogenic HPVs. Other immunization strategies that are being considered include the application of naked viral DNA, viral RNA, vector-based systems or intranasal application of modified HPV protein expressing bacterial strains. Such strategies may have advantages as compared to traditional protein or peptide-based vaccines.

Impact of HPV vaccination in India: To study the prospects of available HPV vaccines, the two HPV vaccination projects were initiated in India. One was a post-licensure observational study for operational feasibility of school-based and community-based vaccination in Khammam district (Andhra Pradesh, Gardasil®) and Vadodara (Gujarat, Cervarix™), conducted by the State Governments in collaboration with Indian Council of Medical Research and PATH (Program for Appropriate Technologies in Health; a US based, non-profit non-governmental organization). No biological outcome is measured; hence it was not a clinical trial. The second was a multi-centric clinical trial to investigate immunogenic efficacy of two doses (6 months apart) compared to the conventional three doses (at 0–2–6 months) of Gardasil®, which if found successful would have resulted in 33% cost reduction. There were media allegations of "vaccine-related" deaths of four girls in Khammam, both studies have been indefinitely suspended by the Union Government. The deaths have since been investigated and confirmed as unrelated to the vaccine. However the skepticism for the need and safety of HPV vaccines in the Indian context continues.

6 Anti-HPV Therapeutic Approaches using Herbal Derivatives

HPV is the principal sexually transmitted infection and currently no specific treatment is available for HPV associated diseases. Also, recently available strategies are primarily anti-cancer but not anti-viral. So, for the magnitude of the disease and relevance to the health need in India, efforts are being made to develop therapeutics for both, elimination of HPV infection and control of HPV activity in already infected advanced stage cervical lesions. Considering low toxicity and the pharmacological safety, potent anti-HPV activity has been demonstrated in some of the herbal derivatives such as curcumin,[40] berberine[57] and Bryophyllum pinnata,[58] which could selectively suppress host transcription factors that are essential for the expression of viral oncoproteins E6/E7, and appear to have potential therapeutic utility for the control of advanced-stage cervical cancer. A recent pilot Phase II clinical trial has demonstrated that treatment with Praneem, a polyherbal formulation (tablet) based on purified extracts of Neem (*Azadirachta indica*) leaves, can remove highly oncogenic HPV Type 16 from the uterine cervix in an overall 80% of cases,[59] while a multicenter clinical trial of other polyherbals such as Basant and Curcumin are in its final stages. In addition, several therapeutic nucleic acids (TNAs) based therapies for cervical carcinoma including ribozymes, small interfering

RNAs (siRNAs) and antisense oligonucleotides (AS-ODNs) have shown effective therapeutic potential against HPV infection.[60] In vitro experiments with TNAs successfully inhibited E6/E7 expression and caused induction of apoptosis and/or senescence in cervical carcinoma cells. Early ribozyme and AS-ODN approaches showed promise as therapeutic moieties for cervical carcinoma. Despite the very high in vitro efficiency of siRNA-based therapies they present the same issues that burdened clinical development of ribozymes and AS-ODNs. These issues include intracellular target accessibility, specificity and delivery. By using combined treatments with multiple targets it will be possible to apply TNAs directly to the cancerous cervix to destroy viral RNA and eliminate the tumor. Radiotherapy and Chemotherapy services are still the mainstay of treatment given to a large proportion of advanced-stage cervical cancer patients. Several new chemotherapy drugs show promising activity for the treatment of advanced or recurrent cervical cancer. Development of new multi-drug chemotherapy treatment regimens that incorporate new or additional anti-cancer therapies is an active area of clinical research. Patients for palliative and curative treatment need to be identified at the beginning of the treatment plan and palliation may be achieved with the minimum maching time. Palliative treatment includes systemic chemotherapy with conventional cytotoxic agents or a combination of cytotoxic drugs and novel targeted biological agents.

7 Micro-RNA New Candidate Target for Prognostic and Therapeutic Strategies

Micro ribonucleic acids (miRNA) are small noncoding RNAs, 18–25 nucleotides in length and regulate the translational efficiency of target mRNAs. More recent studies have emphasized the potential application of miRNAs as biomarkers for detection and progression of several human cancers including cervical cancer. MiRNAs are released and circulated in the blood of cancer patients. miRNA can act as a tumor suppressor as well as an oncogene and changes the expression and the levels of circulating nucleic acids which have been associated with tumor burden and malignant progression. The area of metastasis-associated miRNA markers in relation to oncogenesis is expanding rapidly and these markers have recently been referred to as "metastamirs". A method to reliably predict disease outcome would be important for individualized therapy by identifying patients with high risk of treatment failures before therapy.

Study by XiaoxiaHu et al. revealed that the therapeutic delivery of miR-200a and miR-9 could be a new promising treatment strategy for effective control of cervical cancer.[61] Reduced expression of miR-143 and overexpression of miR-21 were observed in cervical cancer tissue samples suggesting that, the occurrence and progression of cervical cancer might be associated with the deregulation of one or more miRNAs.[62] Mir 21 is frequently upregulated in solid tumors and participates in oncogenic signaling. MiR-21 is transcriptionally induced by AP-1 which is essential for HPV transcription. Another miRNA, miR-29 restrains cell cycle progression and induce apoptosis and promotes malignant transformation induced by HPV.[63] The epithelial cell-specific marker *LAMB3* expression is increased in the presence of the HPV-16 E6 oncogene and this effect is mediated through miR-218. Also Laminin-5 is required in RAS and NF-kappaB blockade induced tumorigenesis of human squamous cell carcinoma and a marker of invasiveness in cervical lesions. MicroRNA-218 (miR-218) can target laminin-5 beta3 (LAMB3), but suppressed by HPV-16 E6 protein. It suggests that single nucleotide polymorphisms (SNPs) in pri-miR-218 and LAMB3 may individually and/or jointly contribute to cervical cancer carcinogenesis.[64] Reduced miR-34a expression is associated with high-risk human papillomavirus (HR-HPV) infection and cervical cancer. Moreover, this reduced expression of pri-miR-34a occurs not only in cervical cancer but also in precancerous lesion even before morphologic change. The inhibition of miR-34a expression induced by HR-HPV E6 in the p53-dependent pathway is probably an early-onset event in the development of cervical cancer.[65,66]

8 Stem Cell Therapy: A Potent Concept for the Treatment of Cervical Cancer

Cancer stem cells (CSCs) or cancer initiating cells are a subset of cancer cells within a tumor that have the ability to self-renew and differentiate into specialized cell types. Their ability to differentiate into multiple cell type is indicative of their potential for tissue repair and organ regeneration. Advances in medical investigation have fostered a deeper understanding of cancer, from tissue anatomy to molecular mechanisms of tumor initiation, progression and development. Recent cancer researches have guided scientists to recognize the central role played by cancer stem cells in sustaining malignancy and chemoresistence.

However, there is no consensus on how cancers are initiated or how they develop from small neoplasms to fatal invasive cancers. Based on

experimental evidence, it has been proposed that these cancer stem cells are the origin of tumors. A small fraction of cells within a tumor is endowed with stem cell-like features that include unlimited proliferative potential and asymptomatic cell division, giving rise to all the other components of the neoplasm that enable the tumor to relapse. This cellular subset, whose numbers may vary in different tumors, can initiate and sustain tumor growth and derive relapse, is called Cancer Stem Cells (CSCs), which are generally insensitive to standard therapies. Stem cell therapy has been termed as a very potent concept for the treatment of cervical and other solid tumors. Despite continuous improvements in cancer management and control, locoregional recurrence or metastatic spread still occurs in a high proportion of patients after radiotherapy or combined treatments. It has been accepted now, that only a small subpopulation of CSCs can cause recurrences and that all CSCs must be killed for permanent tumour cure.[67,68]

This promising new area of stem cell research has brought hope and challenge in the fight against many degenerative disease and cancer. Significant progress has been achieved in hematology, embryology and neurobiology through the isolation and characterization of the cancer stem cells. Several methods exist for isolation and identification of CSCs in human leukemia and several solid tumors on the basis of the expression of cell surface CD markers. However, newer fields such as cervical cancer stem cells are still not well studied. Therefore, a better knowledge of cervical cancer stem cells is crucial in the study and treatment of cervical cancer and the human papillomavirus infection. A study by Kondo et al. showed the existence of a small side population (SP) that is enriched for cells with the characteristics of cancer stem cells in the human adenocarcinoma cell line HeLa.[69] Like other solid tumors, cervical cancer also contains a heterogeneous population of cancer cells. Several studies have revealed that the putative stem cells from solid tumors and cancer cell lines via the capacity to self renew, drive tumor formation. Recently Fenget et al. suggested that CD44+CK17+/ sphere-forming cervical cancer cells display stem cell properties.[70,71] Under optimal environmental conditions these cervical cancer stem-like populations are predicted to show self-renewal, chemoresistance, and *in vivo* tumorigenicity, thereby promoting recurrence of the cancer and possibly metastasis. This experimental system may represent a suitable *in vitro* model to study cervical cancer stem-like cells and challenge them with targeted agents specifically interfering with cervical cancer stem-like cell self-renewal and survival.

From our preliminary study, we found that cervical cancer cell lines contain a subpopulation of tumor initiating cells with stem-like properties that are target population for high-risk HPV infection, thus facilitating the approach to therapeutic strategies aimed at eradicating the tumorigenic subpopulation within cervical cancer along with HPV.

9 Concluding Remarks

Taken together, the available findings highlight the central role of oncogenic HPV types along with associated risk factors are the prime cause of almost all cases of cervical cancer. Cervical cancer can be cured and/or prevented if precancerous lesions are detected early through conventional screening, and treated before it progresses to high grade lesions. It is desired that every woman undergoes cervical Pap-smear screening and/or HPV DNA testing at least once in her lifetime after the age 35 years. Visual inspection with acetic acid (VIA) and Pap-smear tests, being cost effective and easy to access, may continue to be used as a first line of screening in low resource settings but molecular HPV DNA testing as a confirmatory test must be incorporated. Screening must be continued even after HPV vaccination to avoid the development of cancer due to infection with non-vaccine high-risk HPVs, and for those already infected. Reliable and cost-effective screening methods to identify 'high-risk' population along with upcoming anti-HPV therapeutics for the removal of HPV infection at early stages will facilitate better management and control of cervical cancer. Moreover, genotyping of HPV should be performed, which helps to identify types of HPV infection, predicts possible risk of progression and provides a reference for HPV vaccination. Use of low-cost technologies such as paper smear, self-collected urine or vaginal smears could be implemented for HPV detection to screen large populations in low-resource settings. Cost-effective and affordable second generation, single-dose HPV vaccines against a broad range of HPV types with both prophylactic as well as therapeutic efficacy is desirable. HPV vaccines could preferably be given along with other vaccines in early childhood for better acceptance. Use of additional specific biomarkers such as micro-RNA etc. should be employed discriminately and preferably for prognostic purposes.

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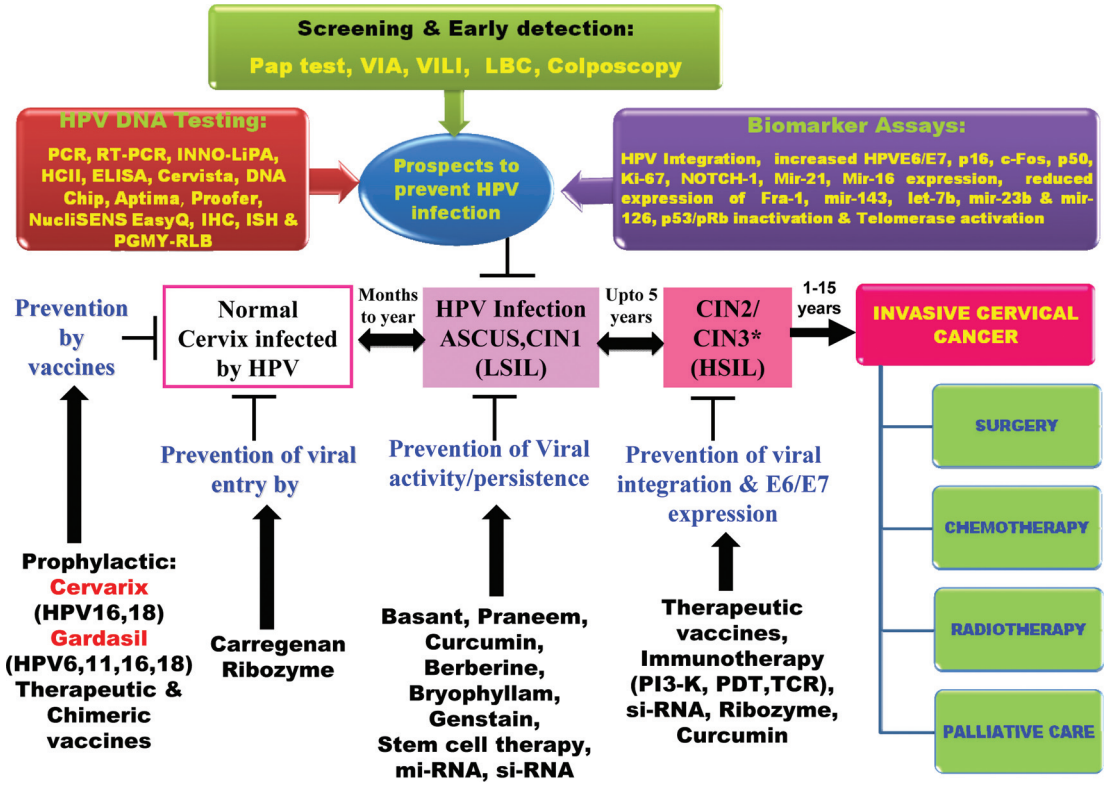

Figure 1: Summary of different preventive and therapeutic approaches for cervical cancer.

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