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REVIEW

Therapeutic Strategies in Advanced Cervical Cancer Detection, Prevention and Treatment

Xolisiwe M Sebutsoe, Nrateng Joyful Nonhlanzeko Tsotetsi, Zodwa Edith Jantjies†, Portia Pheladi Raphela-Choma, Mpho S Choene, Iesetja R Motadi

Department of Biochemistry C2 Lab, University of Johannesburg, Auckland Park Kingsway Campus, Johannesburg, South Africa

†Zodwa Edith Jantjies passed away on July 3, 2024

Correspondence: Iesetja R Motadi, Email lmotadi@uj.ac.za

Abstract: Cervical cancer is ranked the fourth most common cause of cancer related deaths amongst women. The situation is particularly dire in low to lower middle-income countries. It continues to affect these countries due to poor vaccine coverage and screening. Cervical cancer is mostly detected in the advanced stages leading to poor outcomes. This review focuses on the progress made to date to improve early detection and targeted therapy using both circulating RNA. Vaccine has played a major role in cervical cancer control in vaccinated young woman in mainly developed countries yet in low-income countries with challenges of 3 dose vaccination affordability, cervical cancer continues to be the second most deadly amongst women. In this review, we show the progress made in reducing cervical cancer using vaccination that in combination with other treatments that might improve survival in cervical cancer. We further show with both miRNA and siRNA that targeted therapy and specific markers might be ideal for early detection of cervical cancer in low-income countries. These markers are either upregulated or down regulated in cancer providing clue to the stage of the cancer.

Keywords: cervical cancer, miRNA, HPV, vaccine and siRNA

Introduction

Cervical Cancer in Low and Lower-Middle Income Countries

Globally, cervical cancer has been deemed one of the leading causes of cancer-related morbidity and mortality.[1] Cervical cancer is ranked the fourth most common cause of female-related cancer incidence and death; with GLOBOCAN estimating 660000 new cases and 350000 deaths globally, in 2022.[2] The incidence of cervical cancer-related deaths in low-income and lower-middle-income countries (LMICs) remains one of the most recurring causes of death amongst women.[3] The incidence rate is estimated to be 23.8 per 100000 females in low-income regions compared to 8.3 in high-income regions.[3] Geographical variations in the distribution of cervical cancer disease reflects the availability, coverage, and quality of implemented preventative strategies and the prevalence of risk factors.[4] The onset of other major cancers usually develops in later years; however, with cervical cancer, incidences can occur at a relatively young age, this leads to proportionally more life-years lost:[5,6] It tends to develop from the age of 35 years; however, the risk can begin as early as the age of 15 in young girls who are sexually active.[7] This review explores the Human papilloma virus (HPV) life cycle and how the HPV oncoproteins impact disease progression by influencing miRNA expression. With cervical cancer still a challenge in low and lower middle-income countries, we explored possible novel approaches for detection, diagnosis, and therapy to effectively assist towards the control of cervical cancer incidence in these countries.

Human Papillomaviruses (HPVs)

Approximately 90% of cervical cancer cases result from persistent infection with oncogenic HPV strains.[8] HPV is sexually transmitted, which is why cervical cancer is considered a potentially preventable disease.[9,10] There are over 200

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HPV subtypes documented, which are classified as low-risk HPV (LR-HPV) and high-risk HPV (HR-HPV). More than 40 subtypes infect the squamous epithelia of the genital tract.[11,12] LR-HPV is linked with benign neoplasms such as genital warts or mild dysplasia on the cervix.[13] Conversely, HR-HPVs are oncogenic, with the most common subtypes being HPV-16, HPV-18, HPV-31 and HPV-33 which can cause malignant neoplasms such as cervical cancer.[14–17] HPV-16 is the most prevalent subtype, and it is associated with more than 50% of cervical cancer cases.[18,19]

HPVs are small non-enveloped DNA viruses that are 50–60 nm in diameter. The viral genetic material is confined in an icosahedral capsid containing L1 and L2 capsid proteins. The genome comprises of double stranded circular DNA approximately 8 kb that contains 8 genes in total.[12,13,20,21] The genome is divided into 3 functional regions: The first is the upstream noncoding regulatory long control region (LCR) that contains the p97 core promoter together with enhancer and silencer sequences. The second is the early region, which is important for viral replication as well as oncogenesis. It is made up of open reading frames (ORFs), E1, E2, E4, E5, E6 and E7. E1 and E2 are involved in viral transcription and replication. The role of E4 is yet to be elucidated, on the other hand E5, E6 and E7 function as oncoproteins.[20] The third is the late region which encodes the L1 and L2 structural proteins which form the icosahedral capsids for progeny virion generation.[11–13,20]

HPV Lifecycle

HPV infects undifferentiated basal keratinocytes of the stratified squamous epithelium through an exposed microlesion.[22] The HPV life cycle does not possess a lytic phase and therefore relies on basal keratinocyte differentiation to produce virions to complete the infectious cycle.[23] The basal cells in the epithelium are the only cells that proliferate: Basal cells in the epithelium can divide symmetrically to produce more basal cells, or asymmetrically to produce one basal cell and one daughter cell that leaves the basal layer, initiating the differentiation process. The differentiating daughter cells move through the epithelium, acquiring specialised properties until they are released from the source as part of tissue renewal.[24]

HPV infection is initiated with the binding of the major capsid protein, L1 to heparin sulfate proteoglycan (HSPGs) or laminin 5 found on the basal membrane, and this induces conformational changes in the viral capsid.[25,26] Capsid conformational changes expose minor capsid protein L2, together with a conserved site on its amino terminus which can be cleaved by extracellular furin.[27] Cleavage of this conserved site further reveals several protected L2 epitopes, which are important in promoting a second conformational change in viral capsid, allowing the virus to bind to cell receptors, such as α6β4 integrin.[28] This is summarised in Figure 1. The virus is then taken up and internalised through endosomal vesicles into the target cells.[29] The L2 protein involves the retromer and the endosomal virus travels towards the nucleus.[30,31]

The first replicative mode in the HPV life cycle is establishment whereby HPV DNA establishes itself after infection: Following entry into the basal cells, viral genomes settle in the host nucleus as episomes. The early promoter p97 in HPV-16 and HPV-31, p105 in HPV-18 is located upstream of the E6 ORF and directs the expression of early viral genes (E1, E2, E6 and E7) in undifferentiated cells.[33] HPV genome does not express polymerase which is why the early viral proteins E1 and E2 are expressed first, influencing the host cell to progress into the S phase of the cell cycle, thus providing the viral genome with DNA polymerases required for viral replication.[34] The expression of E1 and E2 helps initiate episome replication; HPV episomes are amplified to and maintained at 50–300 copies per cell during the establishment phase.[23,33] Viral genomes are maintained at these low copy numbers, and they replicate along with host cellular DNA.[33]

As the infected basal cells divide, viral DNA becomes partitioned to daughter cells, one of which migrates from the basal layer, initiating epithelial differentiation. The migrating daughter cells induce the productive phase which leads to the activation of the late promoter and the expression of the late viral genes E4, E5, L1, L2, concomitantly increasing E1 and E2 levels. Higher E1 and E2 levels direct the amplification of the viral genome to thousands of copies per cell.[33] The immunogenic capsid proteins L1 and L2 are only expressed in highly differentiated suprabasal cells, resulting in virion assembly and release.[33] The HPV life cycle is depicted in Figure 2.

The expression of viral oncogene during the productive phase does not cause cancer since these cells are bound to be lost from the squamous epithelium. Neoplastic progression happens when viral oncogene expression consistently remains high throughout the epithelium. High-level expression is due to the integration of viral genome.[15] Integration is believed to occur at the E2 ORF, which means E2 is no longer available to act as a negative regulator of E6 and E7 leading to their uncontrolled expression.[35,36] It is important to note that integration is not a normal part of the viral life cycle due to the associated loss of E2, which is important in the synthesis of an infectious virion.[15]

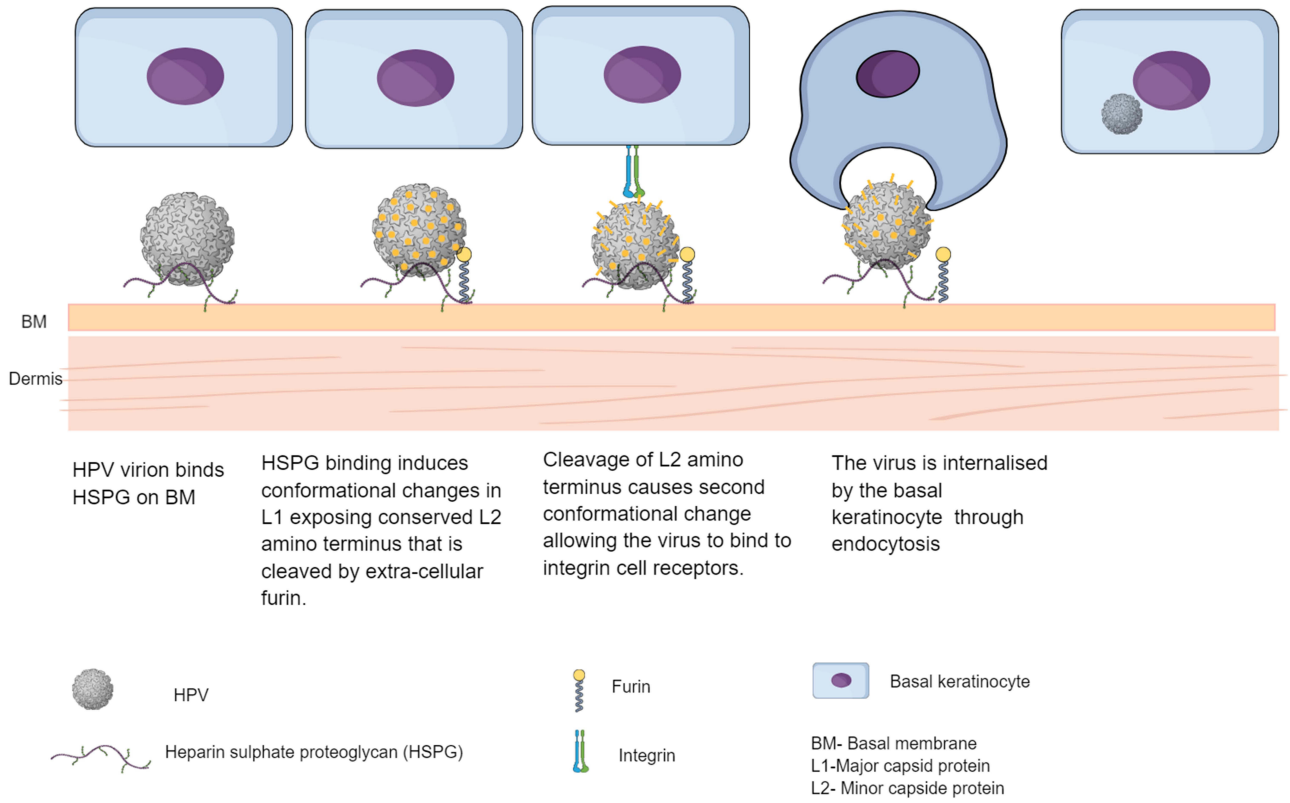

Figure 1 The HPV virion binds to HSPGs or laminin 5 on the basal membrane via L1 capsid protein. This induces conformational changes exposing the minor capsid protein L2. A conserved site on the L2 amino terminus is cleaved by extracellular furin. Cleavage of L2 exposes several conserved protective epitopes of L2 which are critical to infection. The virus is then taken up into the target basal keratinocyte. Data from Roden et al.[32]

Molecular Mechanism of HPV Infection in Cervical Cancer Development

Normal epithelial cells leave the cell cycle upon differentiation; however, E6 and E7 deregulate the standard cell cycle checkpoints, forcing the differentiating cells back into the cell cycle. Furthermore, under normal instances if cells do undergo unscheduled re-entry into the cell cycle, the tumour suppressor protein p53 is activated, which would induce cell cycle arrest and apoptosis of the infected cells. However, E6 suppresses the activity of p53 via two mechanisms: The first is the proteolytic degradation of p53 which is mediated by the ubiquitin ligase E6-associated cellular protein (E6AP). The formation of E6-p53-E6AP tertiary complex replaces the action of MDM-2 (Mouse double minute 2 homologue), a ubiquitin ligase that forms a negative feedback loop with p53 (the activation of p53 induces the expression of MDM-2 which then promotes the degradation of p53).[37,38] In the second mechanism, E6 is involved in the transcriptional repression of p53 by targeting the p53 coactivator CBP/p300.[12] It is also suggested that the E6/E6AP complex also targets other proteins besides p53. These include the tumour suppressors hDlg (human homolog of the *Drosophila melanogaster* Discs large) and hScrib (human homologue of the *Drosophila* Scribble) which may contribute towards cell transformation and oncogenic progression.[39,40] The E6/E6AP complex modulates specific pathways by amplifying the degradation of PDZ (PDS-95, disc-large, ZO-1) proteins giving rise to alterations in cell cycle checkpoints, apoptosis, telomerase activation and supporting the proliferation of infected cells.[12,34,41,42]

Retinoblastoma (pRb) is a tumour suppressor protein that acts as a transcriptional co-repressor preventing entry of cells into the S-phase of the cell cycle through the binding of E2F family of transcription factors.[43,44] Unphosphorylated pRb binds to and suppresses the E2F transcription factors by blocking their transactivation domains and by the recruitment of other co-repressors such as histone deacetylase (HDAC) and SWI/SNF.[45] Phosphorylation of pRb by CDK/cyclins causes the release of E2F transcription factors, allowing the expression of E2F target genes to proceed.[46] In HPV infected cells, E7 binds to pRb marking it for proteolysis,[47] E2F is

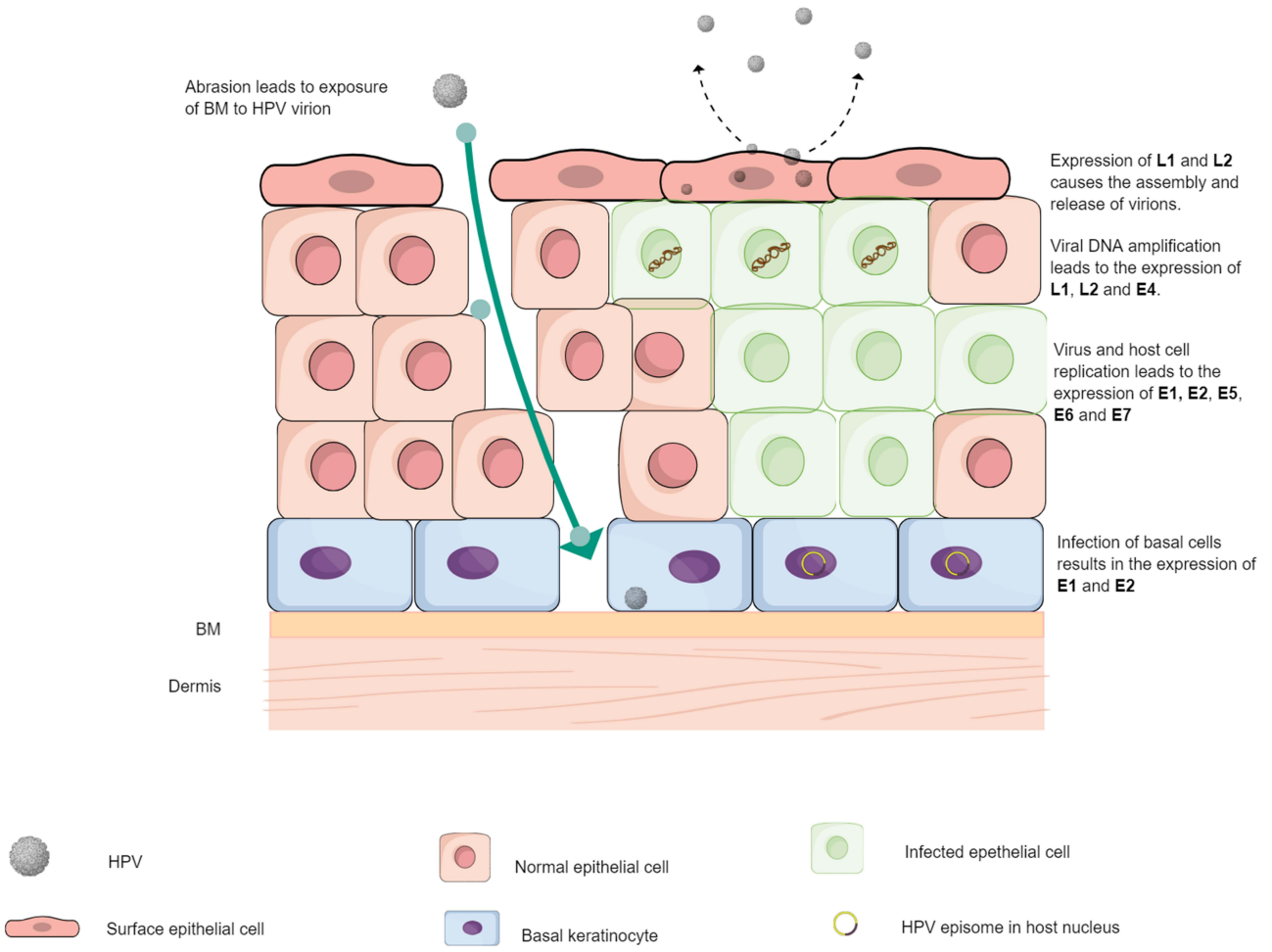

Figure 2 Upon entry into the basal keratinocytes, the viral genome makes its way into the host nucleus as an episome. E1 and E2 are expressed, moving the host cell into the S phase of the cell cycle. As the infected cells divide, one cell remains in the basal layer and the daughter cell migrates upwards as part of cell differentiation. Differentiation initiates viral genome amplification. The expression of E5, E6 and E7 affects numerous metabolic pathways, further promoting proliferation of the differentiated cells. Expression of L1 and L2 only happens in the superficial epithelial cells causing the assembly and release of virions. Data from Roden et al.[32]

released, leading to the irregular activity of E2F transcription factors that promote re-entry into the S-phase. Re-entry into the S-phase provides a replication-efficient environment for differentiating cells which results with uncontrolled cell proliferation.[33] E7 can also target the "pocket proteins" p107 and p130 which are important regulators of E2F.[48,49]

E6 and E7 oncoproteins also interact with other cellular factors that induce genomic instability, tumour progression as well as immune evasion.[34] An increase in cell proliferation rates following the binding and disruption of p53 and pRb proteins by E6 and E7, respectively, leads to cervical intraepithelial neoplasia (CIN).[11] Persistent HR-HPV infection together with other factors that promote cellular transformation may cause gradual progression from mild cervical intraepithelial neoplasia (CIN1) to severe neoplasia with microinvasive lesions and high-grade dysplasia (CIN2 or CIN3) and ultimately invasive disease.[12,50,51]

Persistent infection with HPV causes E5 to mediate and promote phosphorylation of Phosphatidylinositol 3-kinase (PI3K)/AKT and Mitogen-activated protein kinase (MAPK) pathways as well as the EGFR signaling pathway by inducing the expression of vascular endothelial growth factor (VEGF), which a plays a huge role in early angiogenesis of cervical cancer.[52] Furthermore, E5 together with epidermal growth factor (EGF) promote progression of cell cycle arrest by regulating the expression of p27Kip1, a cyclin dependent kinase inhibitor that inhibits the cell cycle.[52,53] In addition, E5 is suggested to play a supportive role in the modulation and immortalization potential of E6, E7 and the hyper-proliferation of cancerous cells.[54] E5 also aids HPV evade the immune system by downregulating (HLA) class 1

molecules involved in antigen presentation of HPV peptides; cells without viral antigen presentation may avoid cytotoxic T lymphocyte attack. Immune evasion contributes towards the establishment of persistent HPV infection and ultimately invasive cervical cancer.[55,56]

Strategies for Cervical Cancer Prevention and Detection

Administration of HPV Vaccines

The primary preventative strategy for cervical cancer is the administration of HPV vaccines. There are currently four prophylactic HPV vaccines available: the first is Cervarix® (GlaxoSmithKline, Rixensart, Belgium) a bivalent vaccine targeting HPV types 16 and 18. The second is a quadrivalent vaccine Gardasil® (Merck & Co., Whitehouse Station, NJ, USA) that targets HPV types 6, 11, 16 and 18. The third is Gardasil®9 (Merck & Co., Whitehouse Station, NJ, USA) a nonvalent, 9vHPV vaccine targeting types 6, 11, 16, 18, 31, 33, 45, 52 and 58. Lastly, Cecolin® (Xiamen Innovax Biotech Co., Ltd., Xiamen, China), another bivalent vaccine targeting HPV types 16 and 18. The first vaccines were approved in 2006 and 2007[57,58] and by the end of 10 years they were implemented in more than 80 countries globally.[59] Gardasil®9 was approved in 2014.[57] Cecolin was approved by the Chinese National Medical Products Administration in December 2019[60] and then prequalified by WHO in 2021.[61,62] The vaccines are based on virus-like particles (VLPs) assembled from recombinant HPV capsid proteins that lack HPV DNA. These VLPs induce specific neutralizing antibodies (nAbs) which can bind to HPV and neutralize it, thus preventing uptake by the target cell.[63] Figure 3 shows the vaccine's proposed mode of action.

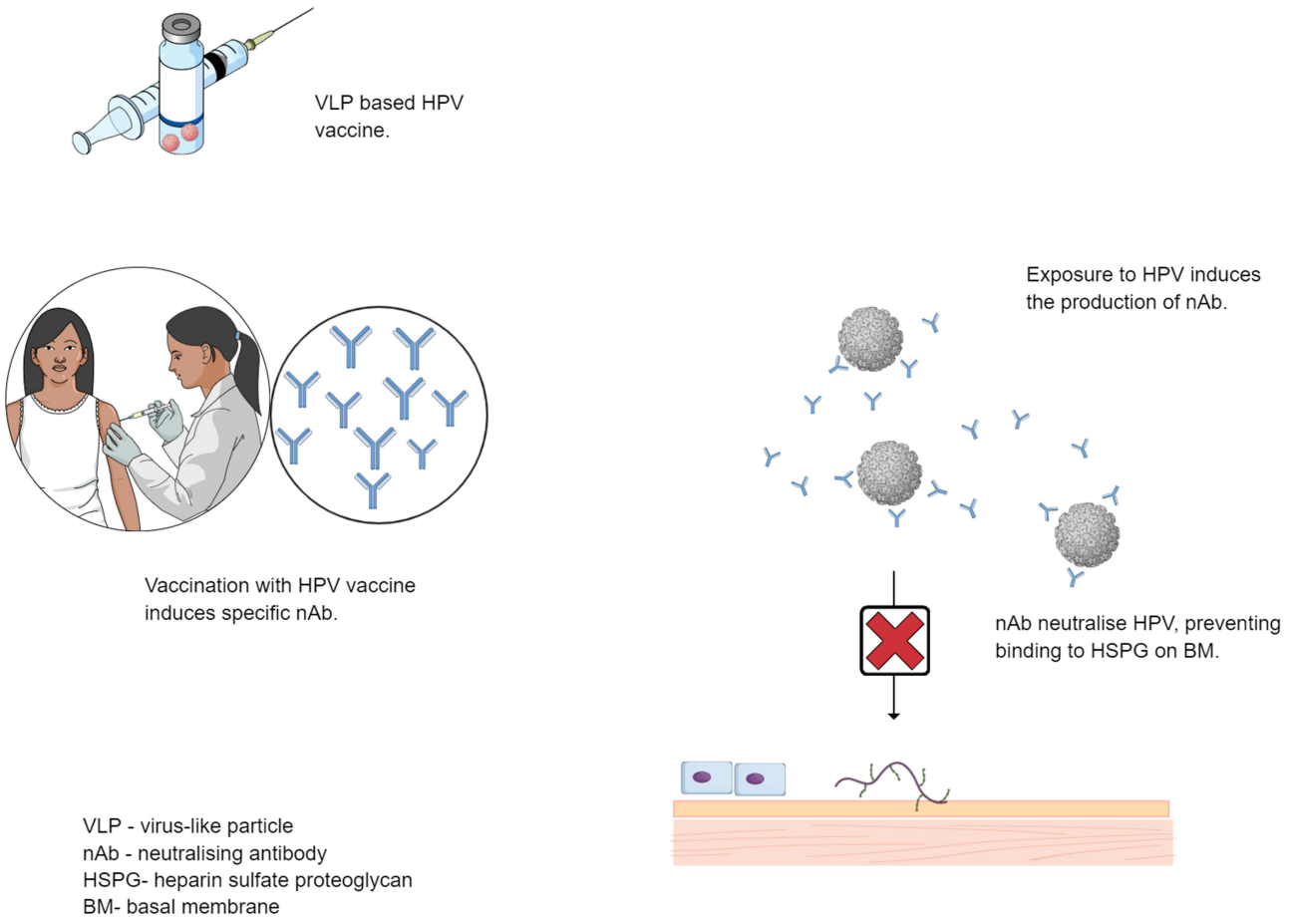

Figure 3 Vaccination elicits an immune response and the production of HPV type specific neutralizing antibodies prevent the HPV virion from binding to the HSPG basal membrane. This prevents uptake of the virus into the basal keratinocytes.

HPV vaccines are mainly targeted towards pre-adolescents and young adolescents, apart from Cecolin which is indicated for women aged 9–45 years.[64,65] For the lower age group, it will take a few years after initial deployment for the cancer preventative benefits to become apparent. However, in low income and LMICs, where cervical cancer incidences are high, the vaccine coverage has been low, with approximately 3% of the initial targeted population of young girls vaccinated by 2014.[66] In 2016, only 14% of LMICs had established vaccination programmes.[67]

Research data has confirmed HPV vaccine safety; however, misinformation has caused vaccination concerns, slowing down the progression of vaccine implementation. Furthermore, most of the LMICs do not have enough resources to initiate HPV vaccine campaigns. Some of the ways to resolve the poor coverage rates would be to issue the right educational interventions, have proper implementation plans as well as a constant supply of HPV vaccines.[68]

The vaccines offer protection against HPV infections, and they induce immune responses significantly higher than those originating from natural HPV infections.[63] However, as it stands, the current prophylactic vaccines prevent HPV entry into the cell, however, for pre-existing infections where cells are already differentiated, the vaccine will not be able to mitigate the progression of HPV infection. To clear infected cells, a cell-mediated immune response would need to be induced.[69] Secondly, since vaccination elicits an HPV-specific type response, the vaccines need a wider range of VLPs in order to provide a wider protection.[63]

Early Detection

Secondary cervical cancer prevention involves screening and diagnosis of precancerous lesions.[70] The main objective of cervical cancer screening is to detect and diagnose treatable precancerous lesions CIN, CIN2, CIN3 and adenocarcinoma (AIS) before progression to invasive cancer.[71,72] This is because early-stage diagnosis has greater survival rates with effective treatment,[73] but outcomes in advanced stages are poor.[74] Moreover, apart from detecting severe abnormalities, screening procedures should also be sensitive enough to allow early detection of HPV infections apart from benign abnormalities. This would help reduce overtreatment and other negatives associated with screening.[72]

Screening methods include traditional pap smear, visual inspection with acetic acid (VIA) and Lugol's iodine (VILI), liquid-based cytology (LBC) and HPV testing.[75] Pap smear has been effective at preventing the development of cervical cancer in developed countries. Nevertheless, factors such as the level of the cytological room, professional technicians, the sampling method, slide quality, dyeing skills as well as cytological personnel experience can possibly affect the effectiveness of the Pap smear.[75] Pap smear results are also subject to interpretation variations between pathologists and sample collection errors, giving rise to false negative results between 15% and 50%.[76,77] Developed countries that have high standard experimental conditions and technical levels, cytology sensitivity can be as high as 80–90%. Contrarily, in regions that have limited resources, sensitivity can be as low as 30–40%.[75]

VIA and VILI are inexpensive HPV screening techniques often used in a low-resource setting. Either acetic acid or Lugol's iodine are directly applied to precancerous cervical lesions obtained from cervical cancer samples. No special equipment or level of expertise is required as results can be visualised by the naked eye both by clinicians and non-clinicians. In a low-resource setting, such results are considered acceptable, sensitive, and cost effective.[78–81]

Unlike traditional Pap smear LBC is a monolayer slide preparation technique that has improved fixation, decreased obscuring factors, and standardized cell transfer. It involves the collection of cervical cells which are then transferred to a sample vial containing a preservative solution as opposed to being immediately fixed onto a slide as in traditional Pap test. This allows for even distribution of the collected sample. A small portion of this sample is used for cytology and the rest can therefore be used for other tests including the HPV test.[81–83] ThinPrep®Pap test (Hologic, Inc, Marlborough, MA) was the first LBC technique to be approved by the FDA.[84]

Screening techniques based on cytology are dependent on the interpretation of morphological changes, whereas HPV testing is based on the detection of HPV DNA, HPV mRNA or related viral markers. HPV testing must be performed under appropriate evidence-based context such as abnormal Pap smear results.[81] Unlike other techniques where analytical sensitivity is crucial, HPV testing does not require high sensitivity as this can lead to a high number of clinically insignificant positives resulting in wrong diagnosis and unnecessary treatments. Unfortunately, the large number of available HPV tests on the market have high analytical sensitivity hence they are the last line of screening.[81]

Early Detection Using Circulating Tumour DNA

Precision oncology is molecular profiling of a tumour to detect any somatic changes that can serve as targets for therapy. Access to tumour tissue for profiling is usually dependent on invasive procedures, for this reason liquid biopsies have become the best option. In oncology, liquid biopsy describes the sampling and analysis of analytes derived from different biological fluids, most commonly blood. The diagnostic information obtained should be the same as one obtained from a tissue biopsy sample.[85,86]

Normal, healthy cells release cell-free DNA (cfDNA) into circulation which results from apoptosis or necrosis. Most of cfDNA in healthy people is released from white blood cells, and bone marrow.[87] Tumour cells also release cfDNA called cell-free tumour DNA (ctDNA) from apoptotic and necrotic cells. ctDNA is considered representative of the entire tumour genome, making it an important component of liquid biopsies.[88,89] Analysis of ctDNA can be used to accurately determine tumour progression, prognosis as well as targeted therapy.[90–92]

Non-Coding RNA

Majority of the mammalian genome, together with that of other complex organisms is transcribed to produce non-coding RNAs (ncRNAs) that are antisense, intergenic, interleaved or overlapping with protein-coding genes.[93–95] It is proposed that ncRNAs account for ~98% of the human genome[96,97] and yet to date, only a small portion has been studied.[98] Although ncRNAs do not code for proteins, they play a regulatory role in the transcription and translation of protein coding genes within the mammalian genome.[94,99]

ncRNAs are divided into multiple classes of varying length and characteristics. Small non-coding RNAs (sncRNA) are 20–30 nucleotides in length,[100,101] examples include microRNAs (miRNA) and small interfering RNA (siRNA). Studies have shown that miRNA and siRNA target genes responsible for various biological pathways, implying an extensive and diverse effect on cellular activity.[98] Conversely, long non-coding RNAs (lncRNAs) have nucleotides greater than 200. lncRNAs can be linear or circular and they are found in the nucleus, mitochondria, or cytoplasm.[98]

miRNA Biogenesis and Cancer

miRNAs are evolutionarily conserved single stranded snRNAs with 21–24 nucleotides. They operate as gene expression post-transcriptional regulators in plant and animals. They bind to complementary mRNA binding sites to induce cleavage or repress translation of target mRNA.[102,103] Most miRNAs are regulated through transcription factors or epigenetic modifications such as DNA methylation and histone modification.[104] To date, approximately half of currently identified miRNAs are intragenic and derived from introns and a few exons of protein coding genes. The rest are intergenic and transcribed independently of a host gene and regulated by their own promoters.[105,106]

The biogenesis of miRNA is categorised into canonical and non-canonical pathways. The canonical pathway which will be discussed, is the primary pathway for miRNA synthesis.[107] RNA polymerase II transcribes miRNA genes, generating long primary miRNA transcripts (pri-miRNA) which are capped, spliced and polyadenylated.[108] While still in the nucleus, the microprocessor complex, consisting of an RNA binding protein DiGeorge Syndrome Critical Region 8 (DGCR8) and a ribonuclease III enzyme, Drosha[109] further processes pri-miRNA: Drosha breaks down pri-miRNA releasing hairpin structured precursor miRNAs (pre-miRNA). Exportin-5 mediates the transport of pre-miRNA into the cytoplasm where Dicer, an RNase II enzyme cleaves pre-miRNA into double stranded miRNA with approximately 22 nucleotides. miRNA is immediately unwound as it interacts with Argonaute protein, retaining one strand to become mature miRNA. The mature miRNA strand is loaded into RNA-induced silencing complexes (RISCs) resulting in the repression of protein synthesis or mRNA degradation.[110] The complementary miRNA strand associates with different Ago protein complexes.[111]

Approximately 60% of protein coding genes within the human genome are regulated by miRNAs at the translational level where they are involved in wide range of physiological processes such as developmental timing, cell proliferation, neuronal gene expression and stem cell division.[112–115] High complementarity is not required for miRNA regulation; therefore, a single miRNA may target multiple mRNAs.[116]

Given their ubiquitous role, it is apparent that any alterations in miRNA expression, sequence or target sites could be the inception of genetic disorders including cancer. Studies have verified the link between the dysregulation of miRNA expression and several types of tumours.[117–120] Genetic changes, such as deletions, amplifications, and point mutations, as well as epigenetic changes, such as histone modifications and irregular DNA methylation, have been linked to aberrant miRNA expression in cervical cancer tissues and cell lines.[121]

miRNAs can also regulate the expression of different oncogenes and tumour suppressor genes.[122–124] Oncogenic miRNAs (oncomiRs), contribute towards malignancy by either upregulating the production of oncogenes or inhibiting the expression of tumor inhibitor genes. These miRNAs are also involved in the development of chemotherapy resistance. On the other hand, tumor suppressor miRNAs (tsmiRs) hinder cancer growth by weakening oncogenes and suppressing cell invasion, cell proliferation and tumour viability.[124,125] Moreover, evidence suggests that viral infections play a substantial role in controlling host gene expression; this is because the exposure to a viral infection triggers changes in miRNA expression.[126] In fact, there are numerous miRNA loci found at HR-HPV-DNA integration sites implying that HR-HPV may influence the expression of miRNA in the host cell.[127,128]

How HPV Oncoproteins Influence miRNA Expression in Tumorigenesis

HPV infections can influence changes in host miRNA expression and encourage tumourigenesis.[129] The HPV type 16 E6 protein has been linked with the downregulation of miR-218 in cervical cancer cells.[130–132] According to Ben et al.[129] E6 caused the expression of miR-218 and miR-27a to be reduced while miR-21 was upregulated. miR-218 is a tumour suppressor miRNA that suppresses the expression of Scm-like with four MBT domains 1 (SFMFBT1) and defective in cullin neddylation 1, domain containing 1 (DCUN1D1) by directly binding to the 3′UTRs of their mRNAs. When SFMBT1 is over-expressed, it induces Epithelial Mesenchymal Transition (EMT), resulting with increased migration and invasion. DCUN1D1 also increases cell migration and invasion when over-expressed, but this is not done through EMT.[133]

β-1,4-Galactosyltransferase III (B4GALT3) is an enzyme that facilitates the generation of poly-N-acetyllactosamine and has a role in tumorigenesis, specifically migration and invasion.[134] Sun et al,[134] found that both B4GALT3 and miR-27a to be upregulated in cervical cancer cell tissue. Furthermore, their investigation demonstrated that the 3'UTR of B4GALT3 has an miR-27a binding site, therefore, miR-27a targets and upregulates B4GALT3. There is additional evidence that miR-27a is also upregulated in other cancers such as pancreatic carcinoma, gastric adenocarcinoma as well as liver cancer.[135–137] On the other hand, Scheibner et al,[138] showed that miR-27a acts as a tumour suppressor in acute leukemia.

In the report by Ben et al,[129] miR-21 was overexpressed under the influence of E6 protein. Overexpression of miR-21 is found in majority of neoplastic diseases and its overexpression in cervical cancer may be associated with HPV16 integration.[139] In cervical cancer, over expressed miR-21 targets tumour suppressor Phosphatase and tensin homolog (PTEN) and it inhibits its activity thus enabling over-expression of matrix metalloproteinases (MMP), MMP2 and MMP9 which promote cellular migration and invasion.[140,141] Over-expression of these MMPs causes invasive cervical cancer. Other tumours suppressor proteins Tropomyo sin 1 (TPM1), and Programmed Cell Death 4 (PDCD4) are also targets of miR-21, their downregulation leads to increasingly invasive and proliferative cervical cancer.[142]

HR-HPV E7 binds to the tumour suppressor protein pRb with higher affinity compared to the LR-HPV E7.[143] E7 causes pRb to degrade upon binding, releasing E2F transcription factor.[144] In the early stages, TGF-β behaves as a tumour suppressor because it induces cell cycle arrest at the G1 phase, this induces cell differentiation or apoptosis whilst suppressing cell proliferation.[145] However, according to Chen et al,[146] after the degradation of pRb, E2F is released and binds to the promoter region of TGF-β, inducing its over-expression. Over-expressed TGF-β is released into the extracellular space, thus activating the TGF-β/Smad pathway. Smad 4 then binds to the miR-182 promoter region, upregulating the expression of miR-182.[146] Upregulated miR-182 is consistent with advanced stages of primary cervical carcinoma. It disrupts cell proliferation by targeting FOXO1 transcription factor.[147] Figure 4 illustrates how E5, E6 and E7 can cause uncontrolled cell proliferation under the influence of miRNA.

Although a specific miRNA may display oncogenic behaviour in one cancer type, it can act as a tumour suppressor in another. This could be due to the ability of miRNA to participate in distinct pathways, thereby having different effects on

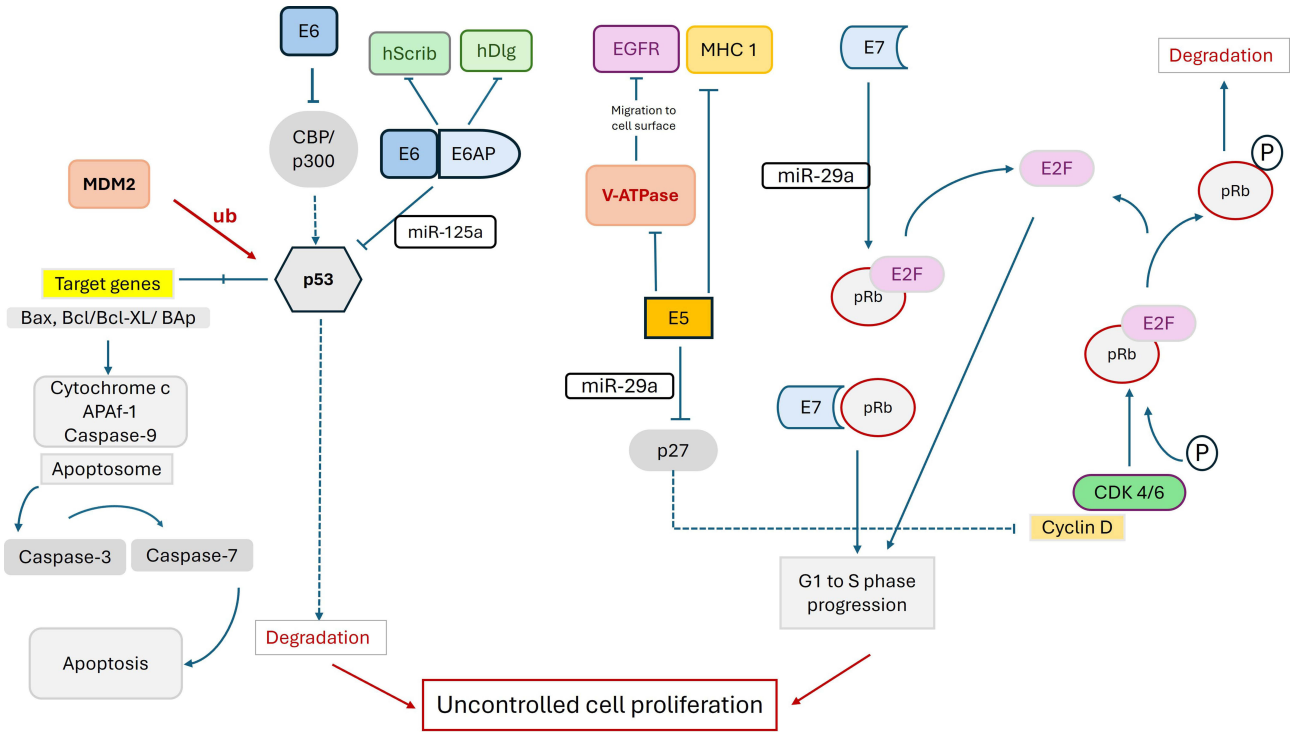

Figure 4 Depiction of the major pathways affected by E5, E6 and E7 that lead to cervical carcinogenesis.

cell survival, growth, and proliferation, which in turn are dependent on the cell type and gene expression.[116] Consequently, miRNA profiling can be a useful and sensitive diagnostic tool for cancer classification.[148,149]

miRNA and Cancer Diagnosis

Studies on different tumours have shown that distinct miRNAs are dysregulated in specific types of human malignancies, which is why they are used in diagnosis, determining the cancer stage, prognosis as well as the response to clinical therapies.[150] Pritchard et al,[151] define miRNA profiling as the measurement of the relative abundance of a specific miRNA group, and this can range from a group of several miRNAs of specific biological interest, to the comprehensive profiling of a given specie. miRNA expression profiling is important in determining miRNAs that are important in the regulation of specific cellular processes. miRNA fingerprints can discriminate different cancer subtypes while also correlating to the specific neoplastic event (for example oncogenic activation).[150] Additionally, miRNAs can be used to help predict the mutational status of important clinical biomarkers.[150]

Some studies have shown that miRNAs are expressed in tissues and secreted into body fluids such as urine, serum, semen, saliva, vaginal fluid, and vitreous humour.[152–155] As such, there is a growing focus on examining of body fluids such as plasma, serum, urine, saliva to determine the levels of circulating miRNAs and to assess their potential use in diagnostic, prognostic, and as predictive biomarkers in cancer. This seems like viable option since it is minimally invasive[116] while being cost effective. What makes this possible is the fact that miRNAs are found to be more stable compared to mRNAs in different specimen types.[151] The relationship between tissue miRNAs and circulating miRNAs in normal population was investigated and the study showed that liver miRNAs strongly correlated with miRNA found in blood. On the other hand, placenta, testis, and brain tissue showed the least correlation between tissue and blood miRNA.[156] Circulating miRNAs have the potential to enhance clinical decision-making and assist in the interpretation of positron emission tomography-computed tomography (PET/CT). This can be done at each patient consultation to evaluate disease response and detect early relapse.[157]

The treatment most cervical cancer patients receive is either chemotherapy, radiotherapy or surgery. Chemotherapy uses chemotherapeutic agents or anti-cancer drugs such as cisplatin to attack cancer cells, however, chemotherapy is not

discriminatory and can also cause damage to normal cells. On the other hand, radiotherapy uses high-energy X-rays to destroy cancer cells, but it also has the potential to damage DNA, exerting more influence on cancer development.[128,158] Cure rates for cervical cancer are between 80% and 90%, the issue arises with the high recurrence rates, which in turn reduce patient survival rates, particularly in the advanced stages.[128,159]

Primary or acquired chemo or radiotherapy resistance remains a major obstacle in the successful management of cervical cancer.[160] It was discovered that resistance is partially caused by the dysregulation of miRNA expression. It is presumed that the up or downregulation of specific miRNAs in cervical cancer can alter the sensitivity towards chemotherapy or radiotherapy.[161] For instance, miRNAs can affect the tolerance to drugs by altering the expression of drug targets and/or enhancing DNA damage responses.[12] The inhibition of oncogenic miRNAs or the substitution of tumour suppressive miRNAs could be used in the development of novel treatments[116] that seek to rectify the tumour's faulty miRNA system.[162]

miRNA and siRNA as Cancer Therapeutic Agents

siRNA is a form of gene therapy made up of a group of double-stranded RNA that is normally 20–24 base pairs long, making it similar to miRNA, and operating within the RNA interference (RNAi) pathway.[163] Double stranded siRNA can be transcribed from genes, infecting pathogens or introduced artificially.[164,165] Both siRNA and miRNA inhibit gene expression post-translationally. siRNAs are entirely complementary to their target mRNA which means their expression is mRNA specific. On the other hand, miRNAs regulate the expression of multiple mRNAs due to partial complementarity to target mRNA; they usually target the 3' untranslated region of mRNA. siRNA regulates genes through mRNA endonucleolytic cleavage. miRNA regulate genes through translational repression or through mRNA degradation, in some rare instances, where miRNA shares high complementarity with mRNA, endonucleolytic cleavage will take place.[166,167]

A significant draw-back with small drug molecules used in conventional treatment is their restriction to certain classes of proteins. Even protein-based drugs that are more specific are dependent on cell-surface receptors or circulating proteins. However, miRNAs and siRNAs can downregulate the expression of any gene and corresponding mRNA transcripts.[167] This versatility is what makes them impressive potential therapeutic agents.

miRNA replacement therapy seeks to restore function of tsmiRs or silencing of oncomiRs through miRNA mimics and miRNA inhibitors.[168] Poudyal et al,[169] investigated a novel miRNA mimic, miR-6852 (miR-SX4) in cervical cancer cells. They found that miR-6852 induced cell cycle arrest at the G2/M phase of the cell cycle and necrosis in HEK293 cells. Song et al,[170] recognised that miR-195 was markedly downregulated in cervical cancer tissue and cell lines. They also identified hepatoma-derived growth factor (HDGF) as the direct target of miR-195 in vitro. Their investigation involved upregulating miR-195 and knocking-down HDGF, which resulted with the inhibition of proliferation, migration invasion of cervical cancer cells. There are clinical studies underway to study the therapeutic potential of miRNAs in different cancers. For example, corbormarsen, a locked nucleic acid-modified oligonucleotide miR-155 inhibitor has been assessed in Phase I (Identifier NCT02580552) and Phase II (Identifier NCT03713320) clinical trials for the treatment of lymphoma and leukaemia.[171] miRNA therapeutic agents are limited due to low stability, possible degradation of naked RNAs by nucleases as well as removal through renal excretion. This is why the efficient delivery of miRNA modulators is also important.[161] Due to limited mRNA complementarity, miRNAs have a broader therapeutic application, unfortunately, this also means off-target effects which can reduce the efficacy of the desired effect. It could also lead to undesired effects resulting from the silencing of functional genes, causing toxicity in cells.[172] This can be overcome by a low-dose mixture of miRNAs that target a single gene with the hope they will work synergistically to reduce the off-target effects.[173]

The specificity of siRNA makes them good candidates for target identification and validation in drug discovery and development.[167,174] siRNA was used to selectively silence HPV E6 and E7 gene expression in CaSKi and SiHa cell lines containing integrated HPV-16 genome.[175] E6 and E7 genes were successfully silenced, and this was sustained for 4 days following the single siRNA dose. Silencing E6 caused the accumulation of p53 as well as the transactivation of the cell cycle control gene p21 and a reduction in cell growth. Contrarily, silencing of E7 prompted apoptotic cell death. HPV-negative cells were not affected by siRNA targeting E6 and E7. Jiang and Milner; Jonson et al,[175,176] sought to silence E6

and E7 to determine growth inhibition in established tumours in a cervical cancer mouse model. Results indicate a reduction in tumour growth as they had hypothesized, with further investigation required for optimal dosing.

Current research is heading towards the association between p53 gene activation, the cytotoxic effect of chemotherapeutic drugs and the silencing of HPV oncogenes with siRNAs.[177] Koivusalo et al,[178] examined the expression of p53 in HeLa cells transfected with siRNAs targeting HPV-18 E6 prior to treatment with carboplatin, cisplatin, doxorubicin, etoposide, gemcitabine, mitomycin, mitoxantrone, oxaliplatin, paclitaxel, and topotecan. They found that silencing of E6 alone did not induce observable apoptosis; however, the combined effect of treatment with E6 siRNA and chemotherapeutic drugs was variable, depending on the compound. They concluded that p53 activation may increase the chemosensitivity of cervical cancer cells.[178] Another research group used siRNAs that simultaneously targeted E6 and E7. The resulting reduction in E6 and E7 expression caused a reduction in cell viability followed by the induction of cellular senescence and an increase in sensitivity towards cisplatin.[179]

At present, there are 3 siRNA-based drugs approved by the food and drug administration (FDA), none of which are cancer related. The first to be approved was Patisiran-210922Orig1s000, approved in 2018 for the treatment of hereditary transthyretin mediated amyloidosis. The second drug, Givosiran −212194Orig1s000 was approved in 2019 for treating acute hepatic porphyria. Most recently, in 2020, Lumasiran-214103Orig1s000 was approved for the treatment of primary hyperoxaluria type 1. There are a few other drugs in late Phase 3 clinical trials.[180]

Limitations of Review / Discussion / Recommendations

An immunocompetent host uses the innate immune response to detect pathogens as the first line of defense. Regarding the initial stages of HPV infection, the host immune response is limited, primarily because the virus infects basal epithelial cells which are guarded from circulating immune cells.[15] This implies that treatment in the initiation stages of infection remains a challenge. Secondly, to know whether the currently deployed vaccines function efficiently despite HPV's evasion of the immune system, we still need to wait a few years. It is also apparent that most vaccination campaigns are targeted towards young girls; however, young boys and men can be carriers of HPV causing further complications in the control of cervical cancer. With cross-infection being a major barrier in HPV control, Zou et al,[181] advocate for HPV vaccination in men as well to help lower infection risk as well as reinfection possibilities in their female partners.

Screening is pivotal to cervical cancer prevention because early intervention provides better outcomes in patients. Apart from technical issues already stated concerning screening, some studies have shown that in low and lower-middle income countries there are individuals who still lack knowledge about cervical cancer and the subsequent need for screening. Furthermore, cultural and religious beliefs pose another barrier whereby lack of spousal and family support resulting from misinformation amplifies the stigma associated with cervical cancer.[182] In some cases, women are reluctant to be examined by male nurses or younger female nurses. Additionally, inferior health infrastructure means there is not adequate privacy during screening, causing further hesitation for women to be screened.[183] Without consistent screening data, apart from women being diagnosed in advanced disease stage, it will also be difficult to know whether the current vaccination strategies are achieving their primary goal. The identification of new diagnostic markers, especially in underdeveloped and developing countries, is highly important. The noncoding RNAs and circulating DNA are providing new hope for early detection of cervical cancer. These markers, as explained in lot of research, can assist with the early detection and treatment of cervical cancer. The approval of HPV vaccine in recent years as part of the therapeutic agents for cervical cancer patients, is another step in the improvement of life in people living with the diseases. The approval for young males to be vaccinated would be another milestone in the fight for cervical cancer. It is paramount that the developing countries speed-up the vaccination of young male as part of the prevention of cervical cancer.

For women already diagnosed with cervical cancer, current treatment options are not discriminatory, causing an array of undesirable side effects. Although options presented in this review are expensive, cancer treatment needs to move in the direction of more targeted approaches. These approaches will offer economical long-term effects because cancer free patients and those in remission are able to contribute to the country's economy. The availability of cancer specific therapy is one such limitation in developing countries, the broad coordination of cervical cancer therapy by WHO would be helpful in making treatment available to developing countries without worrying about cost. This review was limited in its scope to focus only of approved therapeutic agents and future approaches in the early detection.

Conclusions

Low to lower-middle income countries continue to face challenges in reducing or all together eradicating cervical cancer. Vaccine coverage, screening and treatment interventions still require better strategies to reach all members of the population. The current identified miRNA profiling of cervical cancer provides promise to much affordable early detection tool which would surely safely lives. With vaccines now recommended as part of the treatment strategy, this might improve availability of the vaccine as that would lead to more production. We have shown in this review several strategies that might be employed by countries if they were to improve of survival rate of cervical cancer patients. Advanced screening and treatment methods must be sensitive enough to pick up potentially invasive disease while being cost effective and accessible to all hospitals and clinics, including those that are remote and underfunded. Additionally, miRNA profiling is a promising approach towards tailoring patient specific therapeutic strategies.

Ethics Approval and Informed Consent

All research work in this review does not require ethical approval however, each reference work was acknowledged.

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