Review

Androgens in cervical cancer: Their role in epidemiology and biology

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SUMMARY

This comprehensive review delves into the significance of androgens in cervical cancer, examining both epidemiological evidence and the underlying biological mechanisms. Cervical cancer ranks as the fourth most prevalent cancer among women globally, with disproportionately higher incidence and mortality rates in less developed regions where cervical human papillomavirus (HPV) screening remains limited. Recent research highlights the previously underexplored role of androgens in cervical cancer. Notably, cervical tissues house androgen receptors, and elevated levels of endogenous androgens have been linked to an increased risk of cervical cancer. Androgens exert their influence on the development and progression of cervical cancer by impacting key cellular processes, including proliferation, apoptosis, differentiation, and epithelial cell transformation. Furthermore, specific HPV subtypes may interact with androgens, potentially modulating HPV-related cellular degeneration and transformation. In light of these findings, it is evident that androgens assume a crucial role in cervical cancer's pathogenesis. Consequently, further investigations are warranted to deepen our understanding of the intricate relationship between androgens and cervical cancer. Such knowledge advancements can facilitate improved strategies for early prevention and treatment of cervical cancer, especially in regions with limited HPV screening access. This review underscores the importance of considering androgens as a vital component of the multifaceted landscape of cervical cancer etiology and progression, ultimately contributing to more effective clinical interventions.

RESEARCH BACKGROUND OVERVIEW

Epidemiology of cervical cancer and its characteristics

Cervical cancer ranks as the fourth most prevalent cancer among women worldwide, following breast, colorectal, and lung cancers. It results in a staggering 600,000 new cases and 340,000 fatalities annually.[1] There are two primary cervical cancer types: squamous cell carcinoma (SCC) and adenocarcinoma, with SCC accounting for over 90% of cases.[2] Alarmingly, cervical cancer is disproportionately common in low- and middle-income countries (LMICs), where the incidence rate stands at approximately 11.3 per 100,000.[3] Even in high-income nations like the United States, the mortality rate from cervical cancer is twice as high among women residing in high-poverty areas compared to their counterparts in low-poverty regions[4] (Figure 1).

In China, approximately 4.57 million new cancer cases were recorded in 2020, with cervical cancer constituting 2.4% of these cases, affecting around 109,741 individuals. During the same period, cancer-related deaths reached around 3 million, with cervical cancer accounting for 2.0% of this total, resulting in roughly 59,060 deaths (source: International Agency for Research on Cancer [IARC], World Health Organization). While strides have been made in recognizing cervical cancer as a potentially preventable disease, there has been limited improvement in patient survival rates, resulting in a persistently high cervical cancer burden.[5]

Human papillomavirus (HPV) is a primary cause of cervical cancer and is classified as a group 1 carcinogen by the IARC. HPV belongs to a family of viruses comprising over 100 known types, though not all are associated with cervical cancer.[6] Cervical cancer is categorized into several stages: stage 0, often referred to as carcinoma *in situ* (CIS); stage 1, typically subdivided into stages 1a and 1b; stage 2, further divided into stages 2a and 2b; stage 3, indicating cancer spread within the pelvic region; and stage 4, representing advanced cancer (Figure 2, adapted from vagi-wave.co.uk).

Role of androgens in cervical carcinogenesis

Although HPV infection is one of the major risk factors for cervical cancer, other factors, such as genetics, environment, and lifestyle, may also play an important role in the development of cervical cancer. In recent years, studies have shown that androgens also have an impact on the development and progression of cervical cancer.

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Cervix uteri
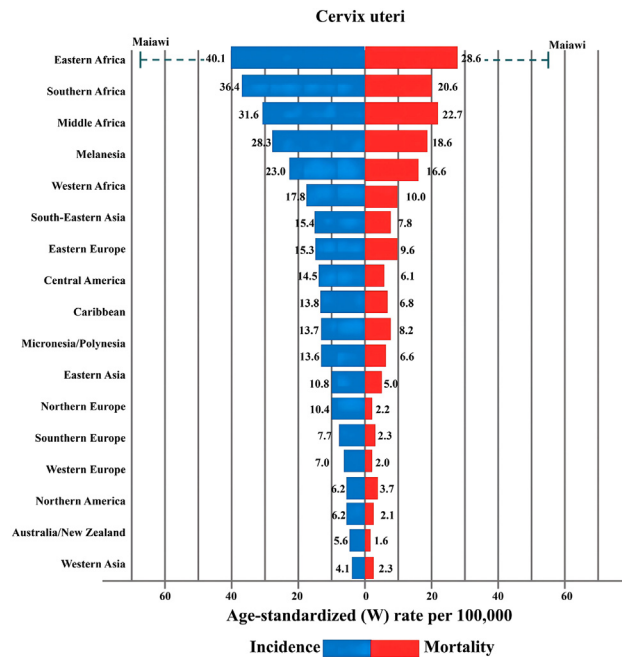
Figure 1. Age-standardized rates of incidence and mortality for cervical cancer in selected regions in 2020
The age-standardized incidence and mortality rates of cervical cancer in 2020 are displayed. The rates are presented as ratios in descending order based on the world age-standardized incidence rate (W). The national rates shown are the highest age-standardized incidence and mortality rates combined.

The androgen receptor (AR) is a nuclear receptor superfamily and is the definitive ligand for androgen. AR regulates the expression patterns of the corresponding genes through "classical androgen/AR signaling"[7] or "non-classical androgen/AR signaling"[8] can regulate the expression pattern of the corresponding genes. Although androgens are male reproductive hormones, women also produce small amounts of androgens, and cervical tissue contains ARs. High levels of endogenous androgens are associated with an increased risk of cervical cancer.[9] AR-dependent signaling affects uterine growth and ovarian function, and AR positivity varies among cervical cancer subtypes. The initial pathological hallmark of cervical intraepithelial neoplasia (CIN) is damage to squamous differentiation, which forms an atypical squamous cell
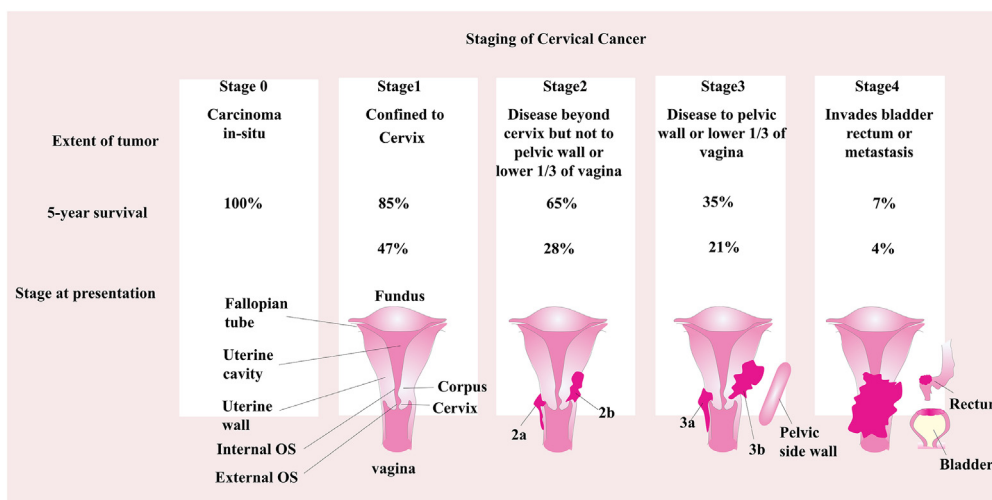
Figure 2. Cervical cancer staging (as quoted from vagi-wave.co.uk)
Illustration of cervical cancer staging. Stage 0: cancer cells are present in the cervix with a 100% five-year survival rate. Stage 1: a small amount of cancer cells is found in the cervical tissue with an 85% five-year survival rate. Stage 2: cancer has spread to the surrounding tissues of the cervix with a 65% five-year survival rate. Stage 3: cancer has spread to the entire pelvic region with a 35% five-year survival rate. Stage 4: cancer has advanced to a late stage and has spread to other organs of the body, such as those near the cervix, including the bladder, rectum, and other distant organs, with a 7% five-year survival rate.
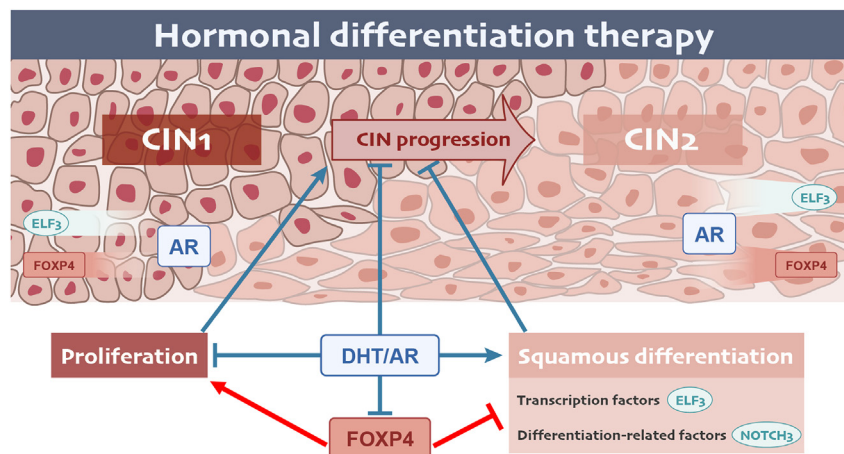
Figure 3. Role of androgens/AR in cervical FOXP4-related proliferation and squamous differentiation
DHT/AR inhibits the functional regulation of FOXP4 (a nuclear transcription factor) and NOTCH3 (a differentiation-related gene) in cervical epithelial cells, promoting cell squamous differentiation and suppressing proliferation, thus promoting the progression of CIN from grade 1 to grade 3.

layer. It has been shown that androgens promote squamous differentiation of atypical cells in cervical intraepithelial neoplasia through an ELF3-dependent pathway (Figure 3).[10]

However, androgens may interact with HPV infection and affect HPV-associated cellular degeneration and transformation. One study found that the E6 and E7 proteins encoded by HPV viruses can interact directly with AR. Indeed, androgen levels may not be associated with cervical cancer and may even be protective.[11] Loss of AR expression is a common event in high-grade squamous intraepithelial lesions and invasive squamous cervical cancer. It may be associated with a complex interaction between high-risk HPV and AR.[12] On the other hand, it has also been found that dehydroepiandrosterone (DHEA) can inhibit proliferation and induce cell death in HPV-positive and HPV-negative cervical cancer cells through an independent mechanism of androgen and estrogen receptors. This study noted that the cell death mechanism in cervical cancer is dependent on the presence of HPV and that DHEA is also highly effective in non-HPV-infected cancer cells.[13,14]

Taken together, the mechanism of action of androgens on cervical cancer remains unclear, and more research is needed to elucidate their specific role. Further research in this area could not only explain why some women suffer from cervical cancer, but also provide new ideas for the prevention and treatment of cervical cancer.

A quantitative study of literature mapping based on CiteSpace

In this paper, a bibliometric approach was used to mine and analyze literature of big data on general trends, distribution, and hotspot changes in the field based on the PubMed literature database, including countries, institutions, authors, keywords, journals, associated diseases, and associated genes. Using cervical cancer and androgen as keywords, we searched the literature from 2013–04 to 2023–04 (Figure 4) and obtained 38 articles, with an average annual publication volume of 4. Among them, the volume of published literature peaked in 2019 with 7 articles; the fastest growth rate was in 2015 with a growth rate of 500%. This indicates that research in this field is growing rapidly and is on a rapid upward trajectory. Globally, the top 21 countries in the field of cervical cancer and androgen research publications are shown in (Figure 5), with the United States of America (14 publications, 36.84%) having the most, followed by China (5 publications, 13.16%) and
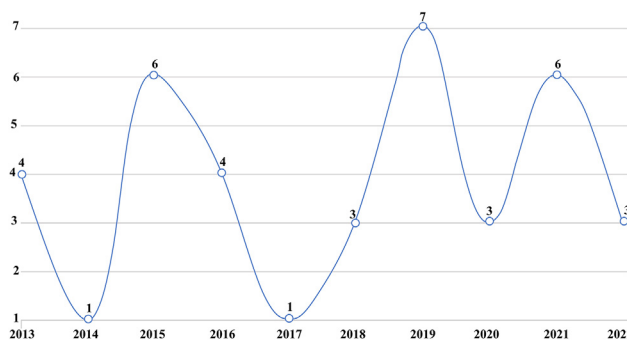
Figure 4. Annual posting trends in cervical cancer and androgen-related literature from 2013–04 to 2023–04
A search was conducted using the keywords "cervical cancer" and "androgen" to determine the number of relevant articles published from April 2013 to April 2023.

Figure 5. Country analysis of cervical cancer and androgen studies (2013–04 to 2023–04)
In the global scope, the top 21 countries in terms of published literature in the fields of cervical cancer and androgen research (the blue area indicates the abundance of articles).

Japan (4 publications, 10.53%). Similarly, the top 20 national research institutions regarding the number of publications for cervical cancer and androgen are shown in (Figure 6). California state university and the University of Szeged published the most papers, with 2 articles, respectively. Amsterdam UMC and the Netherlands Cancer Institute-Antoni van Leeuwenhoek Hospital also published 1 article. Among the 38 publications, the top 30 journals in terms of publication volume are shown in (Figure 7), with the most published journal being Cancer (2 articles), followed by Front Cell Dev Bio (2 articles) and Int J Mol Si (2 articles).
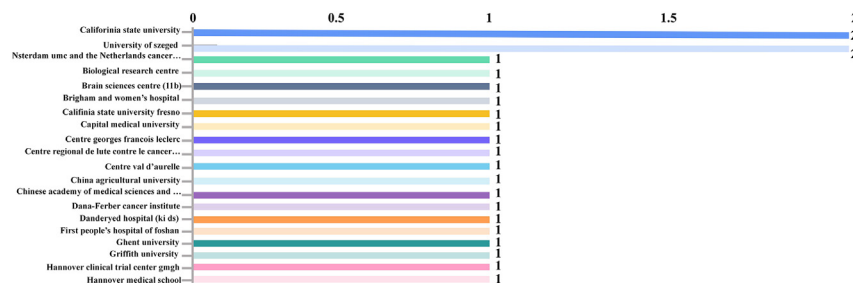
In a search using cervical cancer and androgen as keywords, the top 5 most frequent keywords from 2013–04 to 2023–04 were: androgen, AR, apoptosis, cancer genome, and HPV (Figure 8). In addition, during this search, the entity words of genes in the abstracts of 38 articles were mined and statistically analyzed, as shown in (Figure 9). AR was the most documented gene (18 articles), ESR1 was the second in this field with 11 articles, closely followed by AKT1 with 9 articles.

EPIDEMIOLOGICAL EVIDENCE OF ANDROGENS IN CERVICAL CANCER

### Association between androgen levels and cervical cancer

In recent years, more and more studies have focused on the relationship between androgen levels and the incidence of cervical cancer. Several studies have found that women with higher androgen levels are more likely to develop cervical cancer. For example, a meta-analysis by Castellsagué et al. showed that androgen levels were significantly higher in patients with cervical cancer compared to controls and that androgen levels may be strongly associated with the incidence of this cancer.[15] A population-based case-control study by Zeleniuch-Jacquotte et al. found that women with higher androgen levels had an increased risk of cervical cancer.[16]

In addition to being associated with the incidence of cervical cancer, androgen levels may also affect the prognosis of cervical cancer. Several studies have shown that patients with higher androgen levels have a poorer prognosis. For example, a study by Choi et al. showed that high androgen levels were negatively associated with disease progression and survival in cervical cancer patients by promoting phosphatase and tensin homolog (PTEN) inactivation. This finding suggests that androgen levels may play an important role in the prognosis

Figure 6. Institutional analysis of cervical cancer and androgen (2013–04 to 2023–04)
The research institutions of the top 20 countries with the highest publication output in the fields of cervical cancer and androgen were identified using the keywords "cervical cancer" and "androgen".
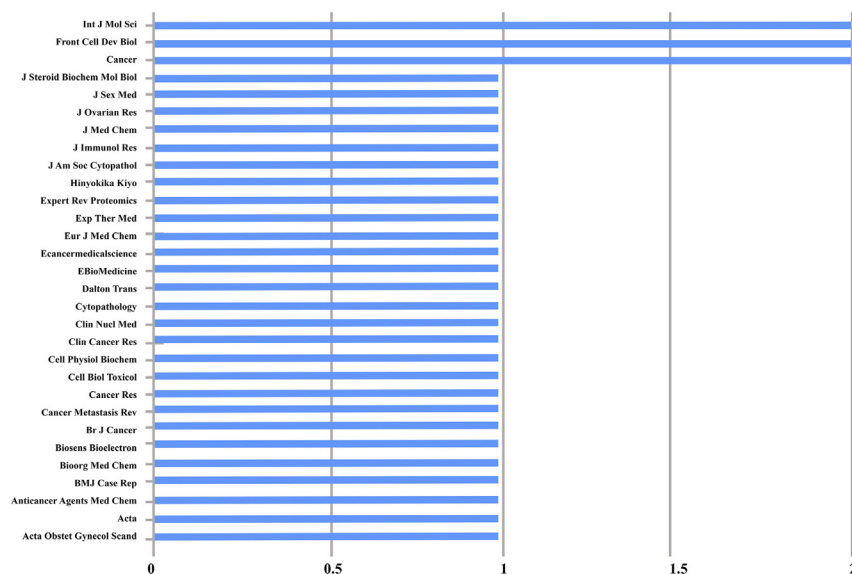
Figure 7. Journal analysis of cervical cancer and androgen (2013–04 to 2023–04)
A search using the keywords "cervical cancer" and "androgen" was conducted to identify the top 30 journal names in terms of publication output.

of cervical cancer.[17] Castellsague et al. examined serum androgen levels in patients with cervical cancer and followed the patients for up to 5 years. The results showed that patients with high androgen levels had a significantly increased risk of recurrence.[18] This result further supports the relationship between androgen levels and the prognosis of cervical cancer.

In addition, there may be a link between androgen levels and the response to treatment in patients with cervical cancer. For example, some studies have suggested that androgens may have an impact on the sensitivity to radiotherapy or chemotherapy. A study by Speers et al. found that AR expression levels in cervical cancer cells were negatively correlated with sensitivity to radiotherapy and chemotherapy.[19] These findings suggest that androgen levels may influence the response to treatment in patients with cervical cancer and thus have an impact on prognosis.

Studies have found an association between androgen levels and the recurrence and metastasis of cervical cancer. Androgens may influence the recurrence and metastasis of cervical cancer through multiple biological pathways. For example, some studies have found that androgens can regulate the ability of tumor cells to invade and migrate. According to Noel et al., the expression of ARs in cervical cancer was negatively correlated with the degree of tumor differentiation and lymph node metastasis.[12] This suggests that androgens may influence tumor recurrence and metastasis by regulating the invasive and migratory capacity of cervical cancer cells. Androgen levels may have a significant impact on the survival rate of cervical cancer patients. For example, Li et al. showed that high androgen levels were negatively associated with disease progression and survival in cervical cancer patients.[20]

Androgen synthesis, peripheral activation, and source pathways associated with cervical cancer

Androgens are a group of biologically active compounds mainly derived from cholesterol synthesis. Cholesterol is converted to androgens through a series of enzymatic reactions in glands such as the adrenal glands, testes, and ovaries.[21] These enzymes include cholesterol side
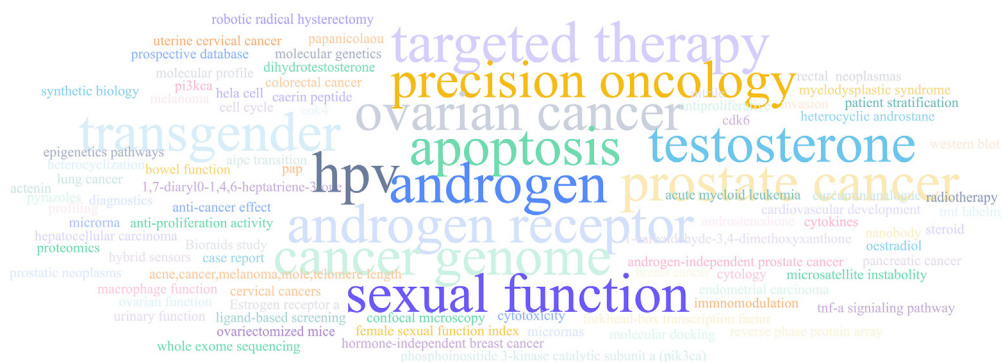
Figure 8. Hot word frequency analysis of cervical cancer and androgen (2013–04 to 2023–04)
A search was conducted using the keywords "cervical cancer" and "androgen" to analyze the keyword frequency in relevant articles from April 2013 to April 2023.
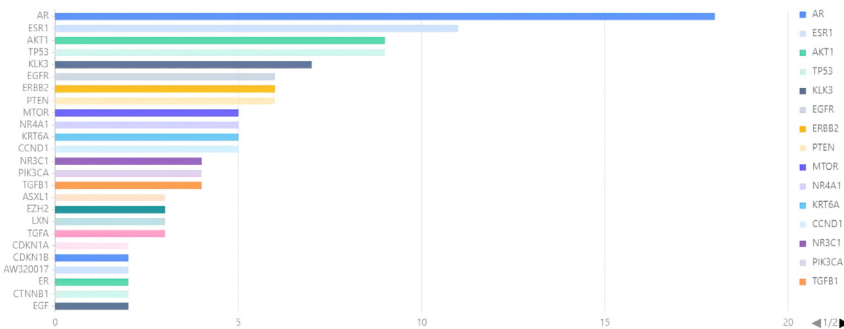
Figure 9. Gene association analysis for cervical cancer and androgen (2013–04 to 2023–04)
Gene entities in the abstracts of relevant articles obtained from the search using the keywords "cervical cancer" and "androgen" from April 2013 to April 2023 were mined and statistically analyzed.

chain cleavage enzyme (CYP11A1), 17α-hydroxylase/17,20-cleavage enzyme (CYP17A1), 3β-hydroxysteroid dehydrogenase (3β-HSD), and 17β-hydroxysteroid dehydrogenase (17β-HSD).[21] The synthesis of androgens *in vivo* is regulated by several factors, the most important of which are growth hormone, gonadotropin-releasing hormone (GnRH), and adrenocorticotropic hormone (ACTH).[22] In addition to being synthesized in the endocrine glands, androgens can be converted to biologically active forms in peripheral tissues through an activation pathway.[23]

Androgen synthesis occurs mainly in the reticular zone of the adrenal cortex and ovaries (see Figure 10).[24,25] Androgen synthesis in the reticular zone of the adrenal cortex proceeds in the classical Δ5 synthesis pathway.[24] In adipose tissue, DHEA, and dehydroepiandrosterone sulfate (DHEAS) are the main sources of androgens. There are other pathways that produce androgens, such as the liver,[25] skin,[26] and salivary glands.[27]

BIOLOGICAL MECHANISMS OF ANDROGENS IN CERVICAL CANCER

Androgen/ARs are associated with cervical cancer stem cells

It is well known that cancer is a heterogeneous disease.[28] This heterogeneity is believed to arise from the presence of a population of cancer stem/progenitor cells (CSPCs) within tumors, which impact cancer phenotypes such as stemness/pluripotency, metastasis, drug resistance, and high recurrence rates, thereby stimulating tumor growth and disease progression.[29] Similar heterogeneity of stem cells is also observed in cervical cancer, where these cells are associated with poor response to chemotherapy/radiotherapy, lymph node metastasis, and pelvic
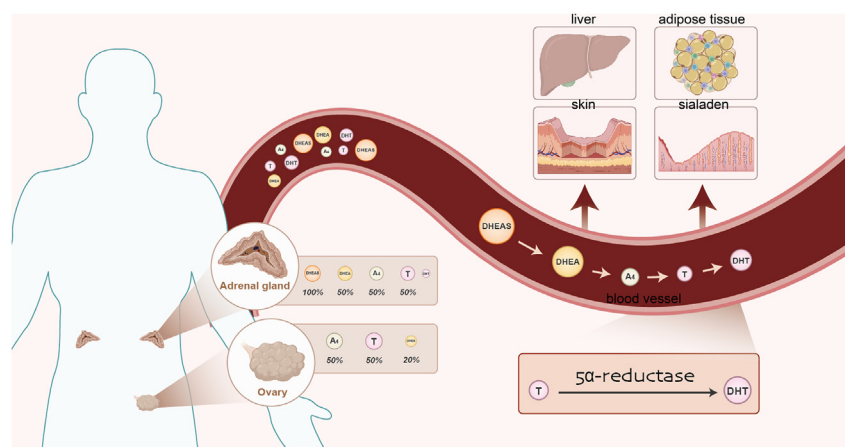
Figure 10. Androgens are mainly produced by cholesterol in the adrenal cortex and ovaries
Androgens are primarily produced from cholesterol in the adrenal cortex and ovaries. In the adrenal cortex, androgens are mainly produced by the cells of the zona reticularis, while in the ovaries, they are primarily produced by the stromal cells and theca cells. The adrenal cortex and ovaries contain varying proportions of DHEAS (dehydroepiandrosterone sulfate), DHEA (dehydroepiandrosterone), A4 (androstenedione), T (testosterone), and DHT (dihydrotestosterone), which are released into the bloodstream. Within the bloodstream, DHEAS undergoes desulfation to produce DHEA, which is then further metabolized through dehydrogenation to A4. A4 is further transformed into T, which can be converted into the biologically active form, DHT, through 5α-reductase. These hormones are released into the bloodstream and transported to the liver, adipose tissue, skin, and salivary glands.
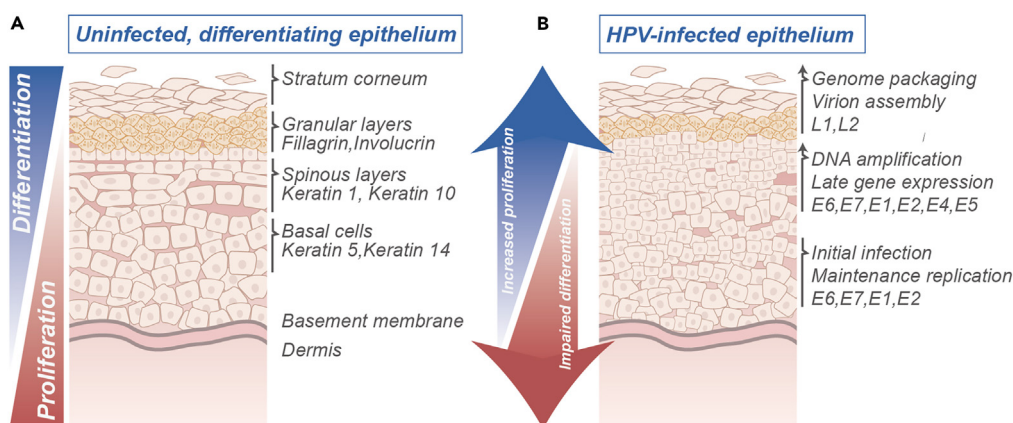
Figure 11. Relationship between cervical epithelial cell differentiation and cervical cancer
Diagram of epithelial cell differentiation in the absence (A) and presence (B) of HPV infection. In uninfected cells, self-renewing basal keratinocytes attach to the basal membrane, forming the basal layer. When keratin 5 and keratin 14 positive basal cells divide, one daughter cell can enter the spinous layer and express keratin 1 and keratin 10. The final differentiation and progression of the granular and cornified layers are marked by the expression of filaggrin and involucrin proteins. Basal epithelial cells generally do not proliferate. In HPV-infected epithelium, proliferation becomes uncoupled from differentiation. Proliferation markers are expressed in basal cells, while gene expression related to differentiation is compromised.

recurrence.[28] Cervical cancer stem cells are a population of cells with self-renewal and multi-lineage differentiation potential and are considered the main cause of tumor recurrence and metastasis. Studies have reported the presence of cancer stem cells in various solid tumors and cultured cancer cell lines, including breast and cervical cancer cell lines.[30]

Various molecules, including ABCG2, ITGA6 (CD49f), PROM1 (CD133), KRT17 (CK17), MSI1, POU5F1 (OCT4), and SOX2, have been reported to be involved in the development of cervical cancer stem cells and serve as markers for these cells.[28] Moreover, the expression of these marker proteins is partly regulated by androgen/AR signaling. ABCG2, also known as ATP-binding cassette (ABC) sub-family G member 2, belongs to the ABC transporter superfamily and mediates the transport of various substrates, including chemotherapeutic drugs, steroids, and xenobiotics, from the intracellular to the extracellular environment through ATP hydrolysis. Studies have shown that increased expression of ABCG2 can enhance the survival of cervical cancer stem cells.[31] Furthermore, it has been demonstrated that ABCG2 is exclusively expressed in cervical cancer stem cells (side population cells) and not in non-side population cells.

In conclusion, the androgen/AR signaling pathway also plays an important regulatory role in the development and progression of cervical cancer stem cells. Researchers can investigate the key regulatory factors of the androgen/AR signaling pathway in the maintenance and function of cervical cancer stem cells, as well as their crosstalk with other signaling pathways, in order to eradicate cervical cancer tumor stem cells further.

Androgen/ARs affect apoptosis and differentiation of cervical epithelial cells

Androgen/AR is initially associated with apoptosis in prostate cancer.[32] During apoptosis, the androgen/AR signaling pathway may regulate the expression of apoptosis-related genes and proteins. For example, androgens may inhibit the expression of Bax and caspase-3 in the apoptotic signaling pathway while promoting the expression of the apoptosis suppressor protein Bcl-2, thereby reducing apoptosis in hair follicle stem cells.[33] We suggest that the androgen/AR signaling pathway may also be associated with apoptosis in cervical epithelial cells. Still, no direct evidence exists for this, and further studies are needed.

The amount of AR was reported to be closely related to the degree of differentiation of endometrial cancer epithelial cells.[34] In addition, AR expression correlated with endometrial cancer type I, early tumor stage (I-II), and low FIGO grade (1–2) and was significantly associated with lymphovascular invasion.[35] Normal differentiation of cervical epithelial cells is important to maintain the normal structure and function of the cervix, and blocked differentiation leads to the development of cervical cancer (Figure 11).[36]

Androgen/ARs are associated with the transformation of cervical cancer

The androgen/AR signaling pathway may play a role in multiple aspects during cervical precancerous transformation. By regulating the proliferation, apoptosis, and differentiation of cervical epithelial cells, this signaling pathway may promote lesion progression.[37] In addition, inflammatory responses are present in precancerous cervical tissues, and the androgen/AR signaling pathway may regulate inflammatory responses, such as affecting inflammatory cell infiltration, cytokine production, and activation of inflammatory signaling pathways.[38,39] These inflammatory responses are closely associated with the development and transformation of cervical precancerous lesions.

Activation of the AR promotes actin and microtubule redistribution, resulting in the formation of pseudopod structures and enhanced migration.[40] In addition, AR overexpression promotes the proliferation and migration of ovarian teratoma (OVCA) cell lines, resulting in a

more aggressive OVCA phenotype.[41] Conversely, low AR expression also leads to a higher risk of tumor brain metastasis. When AR expression was below 10%, the risk of brain metastasis was increased more than 9-fold,[42] suggesting that AR are associated with cell migration properties that affect patient prognosis and overall survival.

A recent study by Fan et al. showed that AR overexpression inhibits the invasive ability of cervical cancer cell lines, which is regulated by miR-130a-3p.[43] The role of the androgen/AR signaling pathway in the invasion and metastasis of cervical cancer may also be closely related to the tumor microenvironment. The androgen/AR signaling pathway may also have some irreplaceable role in the invasion and migration of cervical cancer.

Role of androgens in cervical cancer immunity

HPV is known to trigger chronic inflammation, evade immune surveillance by hiding in keratin-forming cells, suppress cellular immunity, and protect itself with recruited immune cells.[44] However, the immune system is also involved in HPV-induced tumorigenesis.[45] Because of these characteristics, immunotherapy is the best strategy to treat cervical cancer.[44,46,47]

In human bone marrow, AR is widely expressed in stromal cells, macrophages, endothelial cells, myeloid cells, myeloid cells, neutrophils, and megakaryocytes in both males and females.[48] AR was also expressed in both mature CD4$^+$ and CD8$^+$ T cells and B cells.[49,50] In adult mice, androgen deprivation increases T cells in peripheral lymphoid tissue and promotes T cell proliferation and response to antigens.[51]

In addition, AR can exert indirect effects on the immune system through the gut microbiome, as indicated by sex differences in autoimmune diseases.[52,53] The influence of the composition of the gut microbiota on the response to immune checkpoint inhibitors[54] and the efficacy and toxicity of chemotherapeutic agents[55] also need to be taken into account, especially since these efficacy and toxicity show more pronounced differences in women.[56,57]

Of interest is that the interaction between androgens and other hormones may be multi-level and multi-pathway. Studies on the mechanisms of estrogen receptor and AR interactions have shown that androgen-mediated AR signaling is usually antagonistic to estrogen-stimulated proliferation in estrogen receptor-positive breast cancer models.[58,59] Thus, androgens, estrogens, and progestins may regulate cervical carcinogenesis and progression by acting together on key signaling molecules in the cell cycle, apoptosis, invasion, and migration.

Androgen and polyamine metabolism may play a role as cofactors in the development of cervical cancer.[60] Aromatase is an enzyme that converts androgens to estrogens, and obstruction of this enzyme can hinder estrogen formation. Inhibitors designed to target this enzyme induce a dose-dependent decrease in PP2B, nitric oxide synthase 2 (NOS2), and interleukin 6 (IL-6) genes in HeLa cells and regulate the expression of apoptotic genes such as Bax, Bcl2, and caspase-3 at different concentrations. These results demonstrate that the identified aromatase inhibitors are effective against cervical cancer cells and can be used as a scaffold for designing new drugs.[61]

In conclusion, the treatment of cervical cancer is a multifaceted and multidisciplinary process. Combined with the tumor microenvironment, the use of androgens and multiple hormones in individualized combination therapy will become an effective method for the clinical treatment of cervical cancer.

HPV subtypes and the role of androgens

It has been suggested that high-risk HPV types may act synergistically and facilitatively with androgens in inducing the malignant transformation of cervical cancer epithelial cells. The role of androgens in cervical carcinogenesis is well established, but the interaction between HPV subtypes and androgen/ARs has been relatively little studied. This review will synthesize and summarize the latest research results around the interaction between HPV subtypes and androgen/AR, the mutual regulation of HPV infection and androgen in cervical cancer, and new concepts and techniques of cervical cancer-related cancer intervention, aiming to provide a reference for the selection of clinical treatment options.

Interaction of HPV with androgen/AR

The impact of androgens on HPV infection and progression is complex. Studies have shown that higher androgen levels increase the risk of infection development.[62] In addition, androgens can also regulate the expression of HPV genes, and these regulatory processes are closely linked to the development of HPV infection and carcinogenesis. In the course of HPV infection, androgens can affect HPV gene expression in two ways. On the one hand, androgens can act directly on infected cells to increase the replication and transcriptional levels of HPV DNA in cells, thus leading to infection and viral recurrence; on the other hand, it may also disrupt apoptosis and DNA repair mechanisms, thus leading to cellular carcinogenesis. The action of androgens can enhance the expression of HPV-related genes and accelerate the carcinogenesis process.[63]

Regulation of HPV E6 and E7 genes by androgens

Evidence exists reporting that androgens can influence HPV infection and cancer progression by regulating the expression of HPV E6 and E7 genes. Both E6 and E7 oncogenes have multiple cellular targets that promote malignant transformation. Their high transcription is characteristic of aggressive cervical cancer and plays a key role in HPV-induced cervical carcinogenesis.[64]

The findings suggest that HPV-18 E6 is a positive regulator of the AR, and HPV-18 E7 is a negative regulator of the AR through its properties in protein-protein interactions. Further studies revealed that inhibition of AR function by HPV-18 E7 protein could be restored by HPV-18 E6 protein.[65]

Various types of HPV E2 proteins were also found to interact directly with AR and act as major NR coactivators in human cervical cancer cells, including HeLa (HPV-transformed) and C33A (non-HPV-transformed) cells.[65]

In addition, androgens may also affect the expression of E6 and E7 by regulating the expression of microRNAs. A study reported a significant correlation between high miR-130a expression and lymph node metastasis and advanced clinical staging of cervical cancer. Fan et al. found that miR-130a expression was significantly higher in cervical cancer tissues than in healthy cervical tissues. miR-130a can directly bind to the 3′UTR of AR mRNA and regulate HPV-infected cervical cancer E6 and E7 proteins to mediate tumor cell proliferation and invasion.[43] In the presence of the HPV16 E6 oncogene, androgen-regulated miR-218 mediates increased expression of the epithelial cell-specific marker LAMB3.[66] These differentially expressed miRNAs may serve as early molecular markers/targets for disease progression and prognosis, and may represent novel therapeutic targets for miRNA-mediated HPV-induced cervical cancer.

Regulation of HPV L1 and L2 genes by androgens: several studies have shown that androgens can influence HPV infection and cancer development by regulating the expression of HPV L1 and L2 genes. Androgens may inhibit the transcriptional activity of E2 by competitively binding to the transcription factor E2. Since the integration of HPV viruses into the host genome may result in the deletion of L1 and L2 genes, inhibition of E2 activity results in reduced expression of L1 and L2 genes, thereby preventing the formation of HPV viral capsid structures.[67] In addition, androgens may also affect the expression of L1 and L2 genes by regulating the expression of microRNAs.

On the one hand, androgens play a significant role in host defense against HPV infection. When HPV infection occurs, the host immune response is activated, resulting in the secretion of various antiviral proteins, such as interferons and tumor necrosis factors. At the same time, intrinsic and adaptive immune cells, such as helper T cells (Th cells), macrophages, natural killer cells (NK cells), and natural killer T cells (NKT cells), are also activated during HPV infection. These proteins and cells can inhibit viral replication, enhance the cell's antiviral ability, and clear infected cells, thereby preventing sustained viral infection and carcinogenic transformation.[68] However, androgens may weaken these defense responses, promote cell differentiation and proliferation, accelerate abnormal cell proliferation caused by HPV infection, and consequently facilitate persistent infection and malignant transformation of HPV. Studies have found that androgens can induce gender-specific effects on CD8 T cell-dependent antitumor immunity, as they promote CD8 T cell exhaustion and the differentiation of effector T cells, leading to impaired function of CD8 T cells.[69]

It is important to note that although some studies have shown that androgens can regulate HPV gene expression, the exact mechanisms need to be further investigated and explored. Since androgens may have different roles in different types of cells, the mechanisms of androgen regulation may also be different for different types of HPV infections and cancers.

Effect of HPV infection on androgen levels

HPV is a large family, systematically classified into five genera α, β, γ, μ, and ν, comprising 48 species and 206 subtypes. HPV can be classified as low risk (e.g., HPV-6 or HPV-11, causing benign lesions or condyloma acuminata) and high risk (e.g., HPV-16 or HPV-18, causing lesions with a tendency to progress to cancer) according to their association with benign or malignant lesions.[70]

HPV infection can affect the regulation of androgen levels. Studies have shown that in women infected with HPV, patients have relatively low androgen levels. As cervical tumors progress, AR expression decreases, and the downregulation of ARs increases the invasion of cervical cancer cells *in vivo*.[71] Several genes downstream of androgen action in women have a suppressive effect when expressed, the most significant of which is the estrogen receptor, which has a significantly higher expression level in target cells. Therefore, we can conclude that HPV infection further reduces and limits the level of androgenic activity in women.

In addition, some investigators have suggested that HPV viruses can also directly affect the production and transmission of androgenic signals in tissues, leading to altered expression of downstream target genes. For example, in HPV-associated vulvar sebaceous gland differentiated intraepithelial carcinoma, infection with HPV type 16 virus induces the expression of ARs and makes the cells more sensitive to androgens.[72]

In summary, research has shown an inverse correlation between HPV infection and decreased levels of androgens in females, which can impact upstream signaling pathways, such as the expression of ARs. Further investigation is needed to elucidate these mechanisms of influence, in order to better understand the impact of HPV infection on human health and to identify effective preventive and therapeutic approaches.

HPV INFECTION AND THE ROLE OF ANDROGENS IN CERVICAL CANCER
HPV infection and androgens synergistically promote the development and progression of cervical cancer

It has been shown that androgens can interact with the body's immune system and thus significantly affect HPV infection and cervical cancer. First, androgens can increase HPV viral replication and enhance the proliferation, invasion, and metastasis of cervical squamous carcinoma cells. This is because androgens have compensatory mechanisms that can affect cervical cancer development and progression by modulating HPV E6/E7 expression and gene interactions between the fusion protein E2-ATRX, among other pathways, to alter cervical cancer cell signaling, survival, and apoptosis pathways.

A study showed that androgen-positive HPV type 16 CASKI or type 18 HeLa-infected cell lines proliferated faster than in the androgen-positive non-HPV-infected cell line C33A, and that DHEA inhibited the proliferation of all three cell lines at a concentration of 25 mL, with a 40% reduction in apoptosis in C33A cells. In HPV-positive cell lines, higher concentrations of DHEA were required to achieve a similar reduction in inhibition.[73]

Second, the body's natural and cellular immunity continues to suppress and stifle the activity of HPV-infected viruses when HPV infection begins and before it leaves. Studies have shown that androgens can reduce the antiviral potential of T lymphocytes and decrease the immunity of cervical epithelial cells, thereby promoting HPV infection.[69]

In addition, androgens can enhance the signaling pathway of the chaperone protein (c-Met) of hepatocyte growth factor (HGF), thereby promoting HPV invasion in the epithelium and loss of response to stimuli, slowing down the HPV microenvironmental supersystem response. Comparative phosphorylated proteomic analysis revealed that androgens also mediate the interaction of HPV E2 protein with another important protein, THRAP3, thereby regulating HPV reproductive replication.[74] These studies suggest that androgens are an important factor in HPV carcinogenesis.

Role of different HPV subtypes and androgen/ARs in cervical cancer

HPV types contribute differently to the development of cervical cancer. Based on the classification of reactivity associated with the degree of carcinogenesis, HPV types include 13 high-risk (HR) HPV types, including 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68 (IARC groups 1 and 2A), 14 possible high-risk types (HPV 5, 26, 53, 66, 67, 68, 70, 73, 82) HPV 16 is one of the leading causes of cervical cancer worldwide, accounting for more than half of the cases, while HPV 18 is the second most carcinogenic type at 16.5%.[75]

Several studies have shown that certain HPV subtypes are prone to interact with the androgen/AR, which in turn promotes the development and progression of cervical cancer. For example, HPV type 16 may promote cervical carcinogenesis by downregulating AR expression.[76] Deep sequencing of HPV genomic regions led to the conclusion that the HPV16 E7 amino acid is highly conserved among different subtypes of cervical malignancies. This suggests that E7 is critical for HPV16-associated carcinogenesis.[77]

In contrast, HPV18 is closely associated with high expression of AR and may contribute to the development of cervical cancer by enhancing the AR signaling pathway. A global study showed no significant association between HPV18 subtypes and cervical cancer risk or histological type.[78]

In addition, some studies have found that certain HPV subtypes may be unaffected by androgen/ARs and instead promote cervical carcinogenesis and progression through other mechanisms. For example, the HPV31 E7 protein binds to histone deacetylase (HDAC) and specifically activates E2F2 transcription in basal suprabasal keratin-forming cells, promoting replication and thus accelerating tumorigenesis.[79] In addition, whole-genome pyrophosphate sequencing reveals that HPV31 cervical intraepithelial carcinoma grade 2 women are methylated at multiple CpG sites in the E2, L1, and L3 regions, indicating that HPV infection is undergoing transformation.[80] Subtypes such as HPV33 are able to promote cervical cancer development by activating the Wnt/β-catenin or PI3K/Akt signaling pathways to promote the development of cervical cancer.[81,82]

Overall, the differences in the roles of different HPV subtypes and androgen/ARs in cervical cancer are complex and diverse, and further studies are needed to explore their pathological mechanisms. These studies will contribute to a better understanding of the pathological mechanisms of cervical cancer and provide a theoretical basis for the development of relevant therapeutic approaches.

LIMITATIONS OF THE STUDY

From the last half-century, Pap cytology has been considered the standard for continued screening for cervical cancer, with the establishment of the standardized assessment Bethesda system in 1988 and the development of improved techniques such as liquid-based cytology (LBC) and process automation in the 2000s. The role of high-risk HPV in cervical carcinogenesis has been well understood, and its presence in developed cervical cell samples has been detected, so HPV DNA molecular testing is gradually considered a more sensitive tool than Pap smear testing alone. With the increase in HPV infections, especially high-risk types, and the maturation of testing methods, alternative co-tests for HPV and Pap smears and HPV genotyping were introduced. However, considering that HPV testing is associated with high false positives, referral for colposcopy may have psychological side effects, downstream overdiagnosis, and overtreatment. Many factors, such as socioeconomic status, low coverage, lack of relevant health knowledge, poor quality of screening services, and inadequate follow-up management, also seem to hinder the use of cervical cancer screening for optimal outcomes in developing countries.

In developed countries, repeated screening for cervical cancer imposes a significant economic burden on governments and physical and psychological stress on the healthy women who participate in screening. New cervical cancer screening strategies have focused on the use of biomarkers and other molecular tests, including p16/ki67, E6/E7 mRNA, miRNA, and methylation techniques for HPV and host genomes. However, none of these tests can predict the potential population of people with persistent HPV infection who develop cervical cancer, which may be related to host susceptibility, environmental factors, and behavioral patterns other than HPV infection. Modeling risk stratification may yield a comprehensive set of information using patient screening history, while one-size-fits-all protocols are gradually being eliminated by researchers. In addition, multiple regression methods and simple manual learning algorithms have been documented to predict cervical cancer with high sensitivity and specificity. These studies suggest that studies involving large populations, dynamic bioinformatics tools, and comprehensive information warrant further research to develop personalized risk prediction models for HPV persistence and cervical carcinogenesis.

In summary, although current screening strategies have some success, we still need to develop more accurate and reliable methods for cervical cancer screening. For example, HPV integration sites in the human genome can be identified for risk stratification by applying NGS sequencing technology to detect HPV integration into the human genome and measuring HPV E6/E7 oncogene mRNA. Meanwhile, by accurately detecting HPV types in cervical lesions, epidemiologically monitoring the distribution of low- and high-risk HPV types, and identifying new HPV genotypes, we can improve the early diagnosis rate and treatment effectiveness of cervical cancer.

A cervical cancer vaccine is one of the effective measures to prevent cervical cancer. However, vaccination is still controversial, such as the age and frequency of vaccination[83] rather than covering all HPV subtypes.[84] It is expensive and not suitable for women in some poor areas. Therefore, further research and development of a more affordable, reliable, and effective vaccine is needed.

The pathogenesis and molecular mechanisms of cervical cancer are not fully understood, limiting its understanding and treatment. Current research has focused on the relationship between HPV infection and cervical cancer, while other factors remain understudied. Although molecular classification and personalized biomarkers for any HPV variants have been established,[85] more in-depth studies are needed to understand the molecular subclassification of cervical lesions and also to develop precision medicine to better diagnose and treat patients.

FUTURE PROSPECTS

With the increased accessibility of cervical cancer screening and HPV vaccination, the treatment of early cervical disease continues to advance. This progress has led to a decline in the incidence and mortality rates of cervical cancer. Nevertheless, for patients with invasive, advanced, or recurrent cervical cancer, traditional treatments, including systemic chemotherapy, surgery, and combined local radiotherapy, remain largely ineffective. These patients continue to endure substantial suffering and experience low survival rates. Furthermore, while research into host-HPV interaction patterns has made strides, it has yet to yield substantial benefits for patients. Consequently, emerging therapeutic tools, such as targeted immunotherapy, therapeutic vaccines, and androgen therapy, offer newfound hope in addressing HPV-driven carcinogenesis.

Presently, targeted immunotherapy strategies for cervical cancer treatment remain at the experimental stage, with drug research and development primarily limited to laboratory and preclinical studies. Nevertheless, some trials have displayed promise. For instance, the anti-VEGF antibody bevacizumab, when combined with chemotherapy, extended median overall survival by 3.7 months in advanced cervical cancer patients.[86] In a phase II ACT clinical trial, two out of nine patients with metastatic cervical cancer achieved complete regression, though no overall survival impact was reported, suggesting potential for further investigation.[87]

Moreover, the oral tyrosine kinase inhibitor lapatinib has shown promise by enhancing the sensitivity of cervical cancer cells to paclitaxel, reducing tumor microvessel density, and inhibiting neointima formation in tumor tissue.[88] T cell immune checkpoint inhibitors like pembrolizumab, which target the PD-1-PD-L1 axis, have proven to be both tolerable and promising in recurrent or metastatic cervical SCC patients with PD-L1 positivity.[89] These therapies tend to minimize damage to normal tissues by targeting specific molecules. Nevertheless, challenges such as individual variability and drug safety persist. Ongoing research and clinical trials in immunotherapy aim to enhance the survival prospects for a limited number of patients.

Therapeutic vaccines, encompassing tumor cell-based vaccines, live viral vector vaccines, live bacterial vector vaccines, peptide vaccines, and dendritic cell vaccines, may have a pivotal role in controlling cervical cancer's development. These vaccines have the capacity to stimulate the immune system to combat cancers that have already progressed. By eliciting an immune response to tumor antigens, therapeutic vaccines primarily contribute to tumor regression and hold the potential to replace antibody-based drugs in cervical cancer treatment.

Vvax001, a therapeutic alphavirus-based cancer vaccine, is currently undergoing phase I clinical trials.[90] A phase I clinical trial involving 13 individuals with advanced metastatic cervical cancer, utilizing the recombinant live bacterium ADXS11-001, demonstrated a reduction in tumor load in four individuals.[91]

In recent years, several researchers have made notable progress in targeting AR for cervical cancer treatment. Studies have revealed high AR expression in cervical cancer tissues, with this expression level closely associated with clinicopathological features of cervical cancer.[92] Consequently, researchers have explored the treatment of cervical cancer by inhibiting AR expression or function.

Several therapeutic agents targeting AR are presently in clinical trials, including entecavir and bicalutamide. These drugs operate through different mechanisms to inhibit AR activity. Research has shown that these agents significantly inhibit the proliferation and invasion of cervical cancer cells and promote the apoptosis of cervical cancer cells, leading to therapeutic effects. Additionally, some researchers have examined the use of androgen antagonists to treat cervical cancer by blocking androgen signaling pathways. For instance, Abiraterone, an androgen synthesis inhibitor, has displayed effectiveness in cervical cancer treatment.[93]

In summary, the use of androgens or AR treatment for cervical cancer is a subject of great interest. Although these drugs have not yet seen widespread clinical use, available research data suggest that this approach is effective and holds significant potential. As research into androgens, AR, and related signaling pathways continues, it is anticipated that this treatment method will gain wider acceptance and utilization.

With the emergence of novel concepts and technologies for cancer intervention, research into precise cervical cancer prevention, diagnosis, and treatment continues to deepen. Elucidating the molecular mechanisms of HPV persistence and its association with cervical cancer will enable early prognosis prediction for HPV-infected patients. Modern technologies, such as spatial omics and single-cell genomic technology platforms for molecular classification based on HPV integration and genetic profiling, have the potential to facilitate precision medicine. This approach allows clinicians to better allocate medical resources to high-risk patients, reducing the psychological and financial burdens of future cervical cancer screening and HPV vaccination. Given the extensive array of innovative therapeutic approaches in development, the outlook for cervical cancer treatment is promising.

AUTHOR CONTRIBUTIONS

DECLARATION OF INTERESTS

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