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Human Papillomavirus (HPV) infections and cervical cancer: causes, diagnosis and treatment

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Abstract. HPV infections have become the main culprit of thousands of deaths by triggering the development of cervical cancer (approximately 620,000 female cases in 2019, according to the data provided by WHO) due to the oncoproteins E6 and E7 expressed by the high-risk HPV types (for example HPV 16 & 18). To reduce the impact brought, other than physical prevention, three types of prophylactic vaccines- the bivalent vaccine (Ceravix), the Gardasil 4 vaccine, and the newest Gardasil 9 vaccine, were developed for available uses. Diagnosis and screening processes including both molecular diagnosis (DNA & RNA-based diagnosis), and physical diagnosis (including HC2, Cobas, and HR HPV test) are done by patients around the globe in a more common way to eliminate the possibility of delaying treatments (involving cryotherapy and using salicylic acid for treating HPV warts, and cervical cancer treatments including hysterectomy, radiotherapy, and chemotherapy for different stages) until the illness has entered the CIN3 phase. This thesis will focus on the causes, diagnosis methods, current treatments, and possible treatments, of HPV infections and HPV-triggered cervical cancer.

Keywords: Human Papillomavirus, Cervical cancer, cause, diagnosis, treatment.

1. Introduction

Cervical cancer is a type of malignant tumor that elderly women usually suffer from; it is at the same time, a major culprit which caused lots of deaths with a dramatic data of around 342,000 deaths in 2020. The double-stranded type of virus called the Human Papillomavirus (HPV), which is transmitted between individuals by sexual interactions, is the main factor in the majority of all cases [1].

The Human Papillomaviruses that contain circular DNA in their cytoplasm, are classified into two main groups: the high-risk HPVs, and the low-risk HPVs. There are more than 200 HPV genotypes existing: compared to the low-risk HPVs (such as HPV 6, 11, 42, 43, 44, etc.) which normally would only lead to the growth of genital warts, high-risk HPVs (for example HPV16, 18, 31, 33, 35, 39, etc.) are more concerning and significant due to their nature of leading the developments of cancers. To cause cancerous cell mutations and cancer development, these archcriminal high-risk HPVs are required; this is because of the oncoproteins E6 and E7 that they contain. [2]

Oncoproteins E6 prevents and stops apoptosis because it attaches itself to p53 – a tumor suppressor protein, therefore causing the mutated or abnormal cells not to be eliminated by apoptosis that is carried out by lysosomes. In this case, potentially dangerous genetic mutations may accumulate more mutated cells and therefore lead to more mutated cells staying and replicating, meanwhile, increasing the possibility of tumorigenesis -- tumor development.

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E7 oncoprotein affects the normal cell cycle by interacting with the retinoblastoma (Rb), which is a tumor suppressor protein that functions by preventing the development from the G1 phase to the S phase to control the progression of the cell cycle, affecting the normal cell cycle by interacting with the E7 oncoprotein. The binding of E7 acts as an obstacle in this controlled cell cycle, causing it to become uncontrolled while promoting cell division as well. This disruption can cause genetic abnormalities to accumulate and contribute to tumorigenesis [3].

Comparing the current medical developments in the aspects of HPV and cervical cancer, diagnosis methods (screening for infection and cancerous cell growth) are more accessible, accurate, and relatively more reliable than treatment. The current prophylactic Gardasil 9 vaccine and the other preventative vaccines are becoming more acknowledged and commonly used around the globe; however, it is not 100% guaranteed that infections will not be caused. Moreover, the therapeutic vaccines for HPV and cervical cancer are both still under medical research and trials; therefore, diagnosis methods are a lot more mature.

2. Current prevention and treatment

2.1. Licensed HPV prophylactic vaccines – introduction & nature

Currently, the prophylactic HPV vaccine can prevent approximately 90% of HPV infections; popularity has gained significantly in recent years due to an increasing number of vaccinated patients around the globe.[4] Currently, three FDA- (Food and Drug Administration) licensed HPV prophylactic vaccines are available for use, including the bivalent vaccine against HPV 16 and 18 (Ceravix), the Gardasil 4 vaccine against HPV 6, 11, 16, and 18, and the newest Gardasil 9 which prevents HPV 6, 11, 16, 18, 31, 33, 45, 52 and 58 [5].

2.2. Other prevention methods

Since HPV is a virus that is sexually transmitted, there can also be some physical actions done by people to prevent HPV infections. Firstly, contraception should be used and carried out to reduce the probability of exchanging body fluids; HPV testing or screening beforehand can also be beneficial [6]. HPV and Pap tests can be done at the same time, (it is called the co-testing) to detect the presence of both high-risk HPVs and cancerous changes in the cervical cell. Moreover, a testing kit can be used to carry out self-collection to detect the presence of HPV as well [7].

2.3. Genital warts and treatments

More specifically, when focusing on the genital warts that are caused by both high-risk and low-risk HPV infections, there are also methods available to be used to treat this symptom. Mainly two main types of treatment are involved: topical medication (including podophyllin and imiquimod) and physical treatments (such as cryotherapy and laser therapy). Podophyllin acts as a cytotoxic agent which leads to cell disruption and cell death by affecting the mitotic spindles in the cells; imiquimod works by stimulating an immune response towards the infection. Cryotherapy uses liquid nitrogen to freeze the warts grown, and laser therapy uses dense and focused light energy to target and remove the wart tissues

3. The diagnosis of HPV and cervical cancer

3.1. Molecular diagnosis

Two main ways: HPV DNA test, and HPV RNA test. There are three main categories for molecular assays for detecting HPVs in tissues and peeled-off/ exfoliated cell samples by detecting the presence of HPV DNA. The first one is non-amplified hybridization assays, which include Southern transfer hybridization (STH), dot blot hybridization (DB), and in situ hybridization (ISH). Secondly, there are also hybrid capture assays, which are examples of signal-amplified hybridization. Thirdly, the Target amplification assays: These comprise in situ PCR and polymerase chain reaction (PCR), the latter of which is particularly sensitive and specific for detecting HPV.

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On the other hand, for detecting the presence of oncogenic activities in cervical samples, the existence of E6 and E7 oncoproteins of the HPVs will be tested. This will be done by performing nucleic acid sequence-based amplification (NASBA) or by reverse transcriptase (RT) PCR. Single-stranded nucleic acids (for example the viral genomic RNA that is kept in the capsid, mRNA, or even rRNA) are used in NASC Assays.

Currently, for routine cervical screening or testing, there are three DNA-based and one RNA-based assay available for use since they are approved by the US Food and Drug Administration (FDA). The DNA-based assays are the Digene Hybrid Capture 2 High-Risk HPV DNA test (HC2) (with a specificity of 86% and 94.8% sensitivity for females over 20) [8], the Cervista HPV HR test, and the Cobas® HPV test. Besides these assays, the Aptima® HPV assay (RNA-based), which is the only approved RNA-based assay, is also accessible [9].

3.2. Physical diagnosis: screening for cervical cancer

By using both nucleic acid hybridization and signal amplification methods, the HC2 assay can be able to detect and identify more than 10 types (around 13 types, including HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) of carcinogenic HPV types. The HC2 assay is based on hybridizing the RNA probes that contain the complementary base pairs to the genomic base sequence that these 13 types of HPVs contain, in the solution; these are then used to produce cocktails (both low and high) to hybridize HPV RNA and DNA from the samples/ specimens within the solution. Then, antibodies attached to the microtiters which are used to identify specific DNA and RNA will trap the hybrids and let them be fixed in place and stop them from being mobile. By adding and promoting reactions between these immobilized hybrids and the luminescent product added, resulting in the emission of light, whose intensity will be measured by a luminometer to determine the amount of DNA present in the hybrids: the higher the luminant intensity, the more DNA contained [10].

Despite these listed high-risk HPV types that may lead to cervical cancer, by using the Cervista HPV RA test, one more HPV type- HPV 66 can be also tested.

Moreover, the Cobas HPV test can also detect the genomes of several HPV types in a synchronized way by carrying out PCR to amplify around 200 base pairs per time while marking and labeling them with fluorescents as well [9].

For screening and testing the existence of cervical cancer, Pap Smear- the Pap test is now commonly used by governments from lots of countries promoting females between around 25-55 regularly with a regular interval of 3-5 years between the two tests. The samples for Pap tests are taken from the ectocervix and are fixed on the glass slides for further observations by dipping 95% ethyl alcohol. By doing Pap tests, the existence of ASCUS, (atypical squamous cells of undetermined significance, which signals some abnormal changes in the squamous cells but are not severe enough to affirm the presence of cervical cancerous changes or HPV infection) LSIL, (low-grade squamous intraepithelial lesion, suggests mild or low-grade changes in squamous cells, indicating temporary HPV infections but may heal automatically without treatment since these changes are not considered cancerous) and HISL (high-grad squamous intraepithelial lesion, which indicates relatively severe dysplastic changes and shows that the patient is at CIN (cervical intraepithelial neoplasia) 2 to CIN and is very likely to have cervical cancer; treatment should be carried out) [11, 12].

However, due to the fact that there is a high percentage of patients with LISL and ASCUS shown on the Pap test having or at the CIN2 or CIN3 stage, tissue biopsy and other cytology tests should also be done. (In conclusion, Pap tests show higher accuracies when discerning HSIL than LSIL) [13].

Other than the Pap test, Liquid-Based Cytology (LBC) can be done as well to make the test results more reliable. The AutoCytePrep system is one method that can be used for LBC, in this case, cervical cell samples can be obtained and collected in a liquid medium by adding a small volume of liquid to make sure the cell samples can spread more equally; in this liquid medium, squamous epithelial abnormalities can be identified easier because a clearer cellular morphology has been provided.

The ThinPrep system is another method used in LBC. Besides performing the regular steps of LBCs, filtration is also included in this particular system, and this process offers a thinner layer of distributed

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cells that can allow more accurate and clearer detection of cellular abnormalities. From the previous observations, the ThinPrep system has been predicted and concluded to have a higher absolute sensitivity and detection rate since its detection rate of HSIL is higher than conventional screening with similar specificity [14].

HPV test and cervical cytology tests are recommended to be carried out together to increase the precision of the test results; this is called 'co-testing'.

4. Current and possible treatments

4.1. Treating HPV infections

There are currently no antiviral treatments for HPV infections but waiting for the warts to resolve and heal from time to time. However, patients with HPV infections are monitored systematically by doing Pap tests and HPV tests repeatedly to observe any changes in the cervical cells.

However, several methods may be available to cure the warts quicker while improving efficiency. For example, to cure or to remove plantar warts, other than surgical removal, salicylic acid can also be used routinely for a while to chemically remove some extra keratin grown and relatively reduce or cause remission in inflammation. Cryotherapy is another effective treatment that can be used: double-freeze therapy works by freezing the lesion until a 1- to 2-mm ice halo forms, then freezing the lesion again instantly after it has defrosted. It is more painful and risky than using salicylic acid, but it has higher effectiveness. [15].

4.2. Treating cervical cancer

For early-stage cervical cancer, hysterectomy (surgery) is again a major method to treat cervical cancer by removing cancerous body tissues. This can be done by removing both the uterus and the cervix (the Total Hysterectomy), or by removing the uterus, cervix, the nearby tissues, and the lymph nodes caused by metastatic cervical cancer, (the Radical Hysterectomy). Diving deeper into these hysterectomy methods, two surgical interventions including the MIS (Minimally Invasive Surgery) and LACC (Laparoscopic Approach Cervical Cancer) are present. LACC is the typical open surgery that is accessible and suitable for almost all patients. MIC includes removing tumors with smaller incisions which brings the benefits of reducing the level of pain and leading to more rapid recovery; nevertheless, compared to traditional surgeries, MIC can be more costly, can be riskier when removing large-sized tumors, and there might also be technical difficulties faced [16].

Radiotherapy and chemotherapy are the other two major treatments for cervical cancer; radiotherapy is the main treatment for metastatic cervical cancer, while chemotherapy is used for advanced local tumors. Radiotherapy includes External Beam Radiation Therapy- which involves a beam with high energy aiming at the pelvic area directly to kill cancerous cells, and Brachytherapy, which involves placing radioactive materials nearby or inside the tumor with a prescription point of 2cm beside the uterine canal and 2cm above the lateral fornix mucosa. The therapy/ treatment has developed to become more individualized and customized for patients due to the high variability of the location and size of the tumor [17].

Chemotherapy involves taking drugs and medicines to control the spread of cancers through the blood vessels. The drugs that can be used are Cisplatin, Carboplatin, Bevacizumab, Paclitaxel, Topotecan, Docetaxel, and Fluorouracil. By taking these drugs, the cancerous cells will be effectively controlled and killed because the dividing and replicating cancerous cells will be attacked; however, side effects such as hair loss, vomiting, diarrhea, and extra will be very likely to appear. If taking drugs in the long term, the menstrual cycle will be severely affected as well if the uterus has not been removed [18].

5. Possible therapeutic vaccines for HPV vaccine

High-risk HPVs are the major culprits for causing the development of cancers, especially cervical cancers, which have caused 99.7% of cervical cancer developments among all cases. The main factors

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are again the oncoproteins E6 and E7 produced by these HPVs, and these two HPV types caused 70% of cervical cancer among all cases (caused around 690,000 cases); therefore, designing an HPV therapeutic vaccine might be essential to reduce the number of cervical cancer cases [19],[20].

Currently, there are several types of therapeutic vaccines being invented but are still under research and clinical trials, they are cell-based, nucleic acid, live vector, and peptide-based vaccines. Almost all these vaccines aim for the same targets, which are the E6 and E7 oncoproteins (more on E7 since it is more immunologically distinct); APCs (antigen-presenting cells) are intended to be produced by referencing and delivering the antigens on E6, and E7 oncoproteins.

In order to activate CD8+ cells in the immune system, and stimulate an immune response, E6 and E7 antigens must be digested and broken into shorter peptide chains by using proteasomes to form APCs. Also, only the epitopes that can bind and work with MHC molecules will be kept and used to stimulate immune response.

Focusing on live vector-based vaccines that can be classified into either bacterial or viral vectors, they can stimulate strong immune responses due to the nature of how they are highly immunogenic. Nevertheless, safety is not guaranteed, and the efficacy will also be reduced if one vector has been used more than once [20].

Another example is peptide/ protein-based vaccines. Compared to the other types of therapeutic vaccines developed, they are relatively safer with higher stability at the same time; they are also different from the others because lipids or adjuvants are also required in the formation of peptide-based vaccines to boost the T cell responses further. While they are easier to produce, they are also less immunogenic. However, for peptide-based vaccines to work, specific epitopes must also be recognized. (these vaccines are MHC-specific) Therefore challenges/ drawbacks will be brought when mass production starts. [20]

For cervical cancer, cancerous antigens need to be injected into the immune system via the therapeutic vaccine to eliminate cancerous cells. Again, peptide or nucleic acid-based vaccines can also be the main research direction. Peptide-based vaccines introduce virus-like particles again in the form of endogenous DCs; nucleic acid can be brought by the DNA or RNA-based vaccine for further translation and transcription.

The shared main purpose of all possible therapeutic vaccines is to trigger apoptosis of cancerous cells by stimulating cytotoxic T cells to function. The process will start with the DCs brought by the vaccines that will interact with and activate the CD8+ cells that will replicate and proliferate to obtain the ability to carry out cytotoxic functions so that they can be displaced towards the region with inflammation (where tumor cells are located) after leaving the lymphoid tissue.

Eventually, when cytotoxic T cells invade a tumor, they release cytotoxic molecules that cause apoptosis, killing tumor cells that express their cognate antigen [19].

6. Conclusion

With the current medical development, reducing the risk of cervical cancer and HPV infections is possible by accessing the prophylactic HPV vaccines while diagnosis can be carried out using diagnosis and screening methods with relatively high accuracy and sensitivity to prevent delayed treatment and control of the illness; therefore, the possibility of suffering from fatal risks in the terminal stages of cervical cancer also decreases. Instead of controlling and treating cancer physically through surgeries, chemotherapy, or radiotherapy, the invention of a therapeutic vaccine that triggers the CD8+ cells to stimulate an immune response and eventually eliminate cancerous cells by apoptosis is anticipated to thereby cure patients with a higher curative ratio.

References

[1] Tîrziu A, Avram S, Madă L, Crișan-Vida M, Popovici C, Popovici D, Faur C, Duda-Seiman C, Păunescu V, Vernic C. Design of a Synthetic Long Peptide Vaccine Targeting HPV-16 and -18 Using Immunoinformatic Methods. Pharmaceutics. 2023 Jun 23;15(7):1798.

DOI: 10.54254/2753-8818/74/2024.LA19340

[2] Alp Avcı G. İnsan Papillomavirusunun Genomik Yapısı ve Proteinleri [Genomic organization and proteins of human papillomavirus]. Mikrobiyol Bul. 2012 Jul;46(3):507-15. Turkish. PMID: 22951665.
[3] Fennelly C, Amaravadi RK. Lysosomal Biology in Cancer. Methods Mol Biol. 2017;1594:293-308.
[4] Mo Y, Ma J, Zhang H, Shen J, Chen J, Hong J, Xu Y, Qian C. Prophylactic and Therapeutic HPV Vaccines: Current Scenario and Perspectives. Front Cell Infect Microbiol. 2022 Jul 4;12: 909223.
[5] Soca Gallego, Lucia, et al. "Human Papilloma Virus Vaccine." PubMed, StatPearls Publishing, 2021, www.ncbi.nlm.nih.gov/books/NBK562186/.
[6] HPV test-Mayo Clinic. (2024, July 13). https://www.mayoclinic.org/tests-procedures/hpv-test/about/pac-20394355
[7] HPV and HPV testing. American Cancer Society. https://www.cancer.org/cancer/risk-prevention/hpv/hpv-and-hpv-testing.html. Accessed Feb. 15, 2024.
[8] Koliopoulos G, Arbyn M, Martin-Hirsch P, Kyrgiou M, Prendiville W, Paraskevaidis E. Diagnostic accuracy of human papillomavirus testing in primary cervical screening: a systematic review and meta-analysis of non-randomized studies. Gynecol Oncol. 2007 Jan;104(1):232-46.
[9] Tsikouras P, Zervoudis S, Manav B, Tamara E, Iatrakis G, Romanidis C, Bothou A, Galazios G. Cervical cancer: screening, diagnosis and staging. J BUON. 2016 Mar-Apr;21(2):320-5. PMID: 27273940.
[10] Iftner T, Villa LL. Chapter 12: Human papillomavirus technologies. J Natl Cancer Inst Monogr. 2003;(31):80-8.
[11] Sachan PL, Singh M, Patel ML, Sachan R. A Study on Cervical Cancer Screening Using Pap Smear Test and Clinical Correlation. Asia Pac J Oncol Nurs. 2018 Jul-Sep;5(3):337-341.
[12] Mello V, Sundstrom RK. Cervical Intraepithelial Neoplasia. 2023 Aug 8. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan–. PMID: 31335091.
[13] McCrory DC, Matchar DB, Bastian L, Datta S, Hasselblad V, Hickey J, Myers E, Nanda K. Evaluation of cervical cytology. Evid Rep Technol Assess (Summ). 1999 Jan;(5):1-6. PMID: 11925972; PMCID: PMC4781480.
[14] Klinkhamer PJ, Meerding WJ, Rosier PF, Hanselaar AG. Liquid-based cervical cytology. Cancer. 2003 Oct 25;99(5):263-71.
[15] Witchey DJ, Witchey NB, Roth-Kauffman MM, Kauffman MK. Plantar Warts: Epidemiology, Pathophysiology, and Clinical Management. J Am Osteopath Assoc. 2018 Feb 1;118(2):92-105.
[16] Brandt B, Levin G, Leitao MM Jr. Radical Hysterectomy for Cervical Cancer: the Right Surgical Approach. Curr Treat Options Oncol. 2022 Jan;23(1):1-14.
[17] Williamson CW, Liu HC, Mayadev J, Mell LK. Advances in External Beam Radiation Therapy and Brachytherapy for Cervical Cancer. Clin Oncol (R Coll Radiol). 2021 Sep;33(9):567-578.
[18] Cervical cancer chemotherapy | Chemo for cervical. (n.d.). American Cancer Society. https://www.cancer.org/cancer/types/cervical-cancer/treating/chemotherapy.html
[19] Yan F, Cowell LG, Tomkies A, Day AT. Therapeutic Vaccination for HPV-Mediated Cancers. Curr Otorhinolaryngol Rep. 2023;11(1):44-61.
[20] Yang A, Farmer E, Wu TC, Hung CF. Perspectives for therapeutic HPV vaccine development. J Biomed Sci. 2016 Nov 4;23(1):75.