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CRISPR/Cas9 Gene Therapy Innovation: Targeting HPV16 Oncogene Gene for Cervical Cancer Prevention

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Abstract

Human Papillomavirus (HPV) is a causative agent of cervical cancer. Approximately 60% of cervical cancers are caused by the infection of HPV-16. Cervical cancer is the second most common cancer affecting women. Various treatments and therapies are being explored to treat cervical cancer, but there is still a need for safer, more effective, and affordable technologies. CRISPR-Cas9 is a modern biotechnology technology derived from bacterial immune mechanisms to combat viruses and phages. This mechanism can be applied as a therapeutic medium, as it allows for specific and precise genome editing. The aim of this literature review is to determine the potential and effectiveness of CRISPR-Cas9 as a therapeutic agent for cervical cancer. The method used involves a literature study, based on searches on official websites such as Pubmed, Google Scholar, and other official journals with the keywords "CRISPR-Cas9" and "Cervical Cancer." The results indicate that CRISPR can target the Cas9 protein to deactivate the oncogenes E6 and E7 in HPV-16, thereby promoting cell cycle arrest and apoptosis. Various studies have been developed to improve the side effects associated with CRISPR-Cas9. However, research on CRISPR-Cas9 has shown a reduction in cancer tissue and increased apoptosis. This method is effective for cancer treatment and needs further investigation to reduce the incidence of cervical cancer.

Keywords: *CRISPR-Cas9; Human Papillomavirus (HPV); Kanker Serviks; Protein onkogen E6 & E7.*

Abstrak

Human Papillomavirus (HPV) merupakan agen penyebab kanker serviks. Sekitar 60% kanker serviks disebabkan oleh transfeksi virus HPV-16. Kanker serviks merupakan penyebab kanker kedua terbanyak yang menyerang wanita. Berbagai treatment dan pengobatan diupayakan untuk mengobati kanker serviks, tetapi masih dibutuhkannya teknologi yang lebih aman, efektif dan murah. CRISPR-Cas9 merupakan teknologi Bioteknologi modern yang berasal dari mekanisme imun bakteri dalam melawan virus dan faga. Mekanisme ini ternyata dapat diaplikasikan sebagai media therapeutic, karena dapat melakukan pengeditan genom secara spesifik dan presisi. Tujuan dari kajian literatur ini adalah untuk mengetahui potensi dan efektifitas CRISPR-Cas9 sebagai agen terapi untuk Kanker Serviks. Metode yang digunakan melalui Studi literatur, berdasarkan pencarian pada website resmi, seperti Pubmed, Google Scholar, serta jurnal resmi lainnya dengan kata kunci "CRISPR-Cas9", dan "Kanker Serviks". Hasil penelitian menunjukan bahwa CRISPR mampu menargetkan protein Cas9 untuk menonaktifkan onkogen protein E6 Dan E7 pada Virus HPV-16, sehingga mendorong penangkapan siklus sel dan apoptosis. Berbagai penelitian dikembangkan untuk memperbaiki efek samping akibat CRISPR-Cas9. Namun, penelitian CRISPR-Cas9 banyak menunjukan penurunan jaringan kanker serta apoptosis. Metode ini efektif untuk pengobatan kanker, yang perlu dikaji lebih lanjut agar dapat menurunkan angka insidensi kanker serviks.

Kata Kunci: CRISPR-Cas9; Human Papillomavirus (HPV); Kanker Serviks; Protein onkogen E6 & E7.

INTRODUCTION

Cervical cancer is one of the biggest threats to women's reproductive health globally. This disease is ranked second as the leading cause of cancer death in women worldwide. Data shows that every year, about 500,000 women are diagnosed with cervical cancer and 270,000 of them die [1]. Cervical cancer generally arises as a result of *Human Papillomavirus* HPV infection [2-4]. HPV16, as one of the high-risk HPV genotypes, has a significant role in the development of various types of cancer, especially cervical cancer. HPV16 infection often occurs in conjunction with chronic inflammatory conditions such as lichen sclerosis, increasing the risk of neoplasia, not only in the cervix, but also in other genital organs such as the vagina, vulva, anus, and penis. Despite this, cervical cancer remains the most serious manifestation of HPV16 infection [5]

High-risk persistent HPV infection in cervical basal cells leads to the integration of the viral genome into the host genome. The expression of the E6 oncoprotein results in the inactivation of the tumor suppressor gene p53, triggering uncontrolled cell proliferation and neoplastic transformation [6,7]. Thus, inhibition of E6 expression could be a promising therapeutic approach for cervical cancer. The specificity of E6 expression in cervical lesions indicates that targeting of this molecule may be a selective therapeutic strategy, minimizing side effects in normal tissues [8].

CRISPR-Cas9 is a genetically engineered nuclease system, enabling precise genome manipulation. The system utilizes sgRNA as a guide to direct the Cas9 enzyme to a specific location on the genome, where it then induces double-strand DNA termination [9]. The process of DSB repair through non-homologous end joining often results in mutations in the form of nucleotide base insertion or deletion. These kinds of mutations, especially if they occur in protein-coding regions, can interfere with gene function and lead to loss of gene expression [10]. Although CRISPR-Cas9 offers great potential in targeting the E6 gene in cervical cancer, the main challenge lies in the development of a safe and efficient vector for the delivery of the CRISPR-Cas9 system into tumors in vivo.

CRISPR/Cas9 is an adaptive immune system found in bacteria. This system functions as a 'molecular scissors' that cut the DNA of the invading virus, thus preventing viral replication [11]. Compared to other genome-editing technologies such as TALEN and ZFN, CRISPR/Cas9 offers significant advantages in terms of production costs. This is because the main component of CRISPR/Cas9, the Cas9 enzyme, is a protein that can be mass-produced in a simple biological system. The purpose of this paper is to determine the potential for innovation in CRISPR/Cas9 gene therapy with dual sgRNAs targeting the E6 and E7 genes in the HPV16 virus

RESEARCH METHODS

The literature review was prepared using the literature review method. This study uses a literature review approach by conducting a comprehensive search of various scientific databases, including Google Scholar, PubMed, ClinicalKey, and PLOS One. Keywords used in searches included "*Cervical cancer*", "CRISPR/Cas9", "Oncogene proteins E6 & E7 and "HPV". The selected scientific article is a full-text article relevant to the research topic, from an internationally accredited scientific journal.

RESULTS AND DISCUSSION

Mechanism of Cervical Cancer

Cervical cancer is a cause of cancer caused by HPV virus infection, with the most predominant strains being HPV 16 and HPV 18. The HPV genome consists of two main parts, namely the E part, which functions in the viral replication process, and the L part

which functions for the capsid synthesis process to form virulent. The genes that encode this cancer-causing oncogene are E6 and E7. The E6 gene works by inhibiting the tumor suppressor pathway p53, which plays a role in cell cycle regulation and apoptosis, as well as blocking the RIG-I signaling pathway, which is part of the body's immune defense mechanism [12]. Meanwhile, the E7 gene binds to the retinoblastoma (Rb) protein and deactivates it, causing uncontrolled cell proliferation [13]. In the process of infection, HPV DNA can be integrated into the host cell's genome, resulting in uncontrolled oncogene expression, leading to cervical dysplasia and potentially developing into squamous cell carcinoma or cervical adenocarcinoma. In addition, cervical cancer caused by HPV often has an immune evasion mechanism, in which the virus increases the expression of the basic markers CD55 and CD71, allowing cancer cells to survive the body's immune system as well as increase proliferation, migration, and resistance to therapy [14].

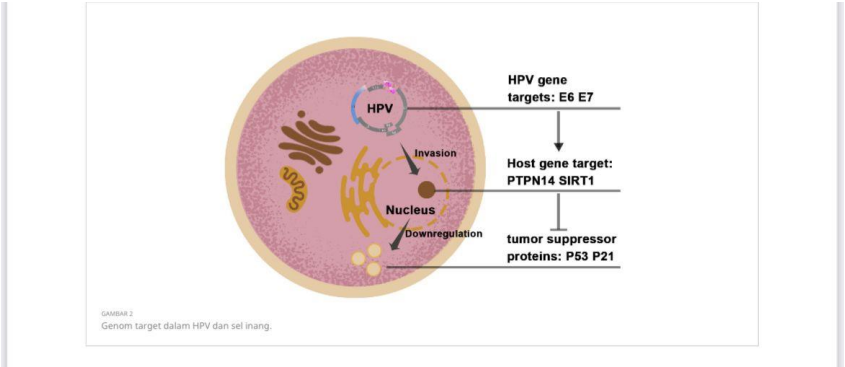

Figure 1. Interaction between the HPV virus and its host cells

Mechanism of CRISPR-CAS Technology

Clustered Regularly Interspaced Short Palindromic (CRISPR) and Cas9 Protein are the latest modern biotechnology technologies that are being intensified because they are attracting a lot of attention. This technology is used to perform gene editing or gene editing more quickly, and precisely [15]. CRISPR/Cas9 is able to make cuts to target DNA through a complex mechanism guided by RNA (ribonucleic acid), by altering, deleting or inserting parts of the target DNA [16]. CRISPR/CAS9 occurs naturally in the bacteria's defense mechanism in protecting themselves against foreign DNA and phages, so it is used as a bacterial immune system in fighting viruses or infections [17].

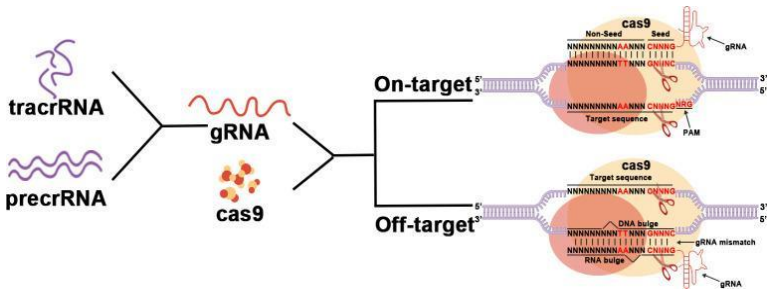

Figure 2. The mechanism of CRISPR-Cas9 in bacteria [18].

The mechanism that occurs in bacteria in fighting viral infections goes through a fairly complex process. Foreign DNA by CRISPR-Cas9 will undergo breakage into

double strand breaks (DSBs) by the Cas9 protein which acts as a nuclease enzyme, which is directed by sgRNA [19]. This *Single Strain* RNA (sgRNA) is secreted by bacteria whose structure resembles the DNA of viruses that infect bacteria [20]. The mechanism that occurs is that after the viral DNA enters the bacterial cell, it will be fragmented into several DNA fragments called Protospacers. The protospacers will be separated and arranged to form CRISPR with the help of Cas 1 and Cas 2 proteins. These two proteins function as endonuclease activity and direct the insertion of Proto Spacers between repeating fragments [21].

After the protospacer is composed into CRISPR, CRISPR will be transcribed into precrRNA that matches the target gene in the virus, and the upstream locus of CRISPR when transcribed will produce tracRNA. preCRNA And tracRNA have homologous fragments because tracRNA can bind to precRNA and form precRNA/tracRNA complexes. When these two components are mature, they are known as gRNa [22]. gRNA functions to bind to the Cas9 protein And will then direct Cas9 to the target DNA to break the bond that produces DSB. After being broken into DSB, it is then simplified again into a distinct Cas structure. This disconnection is in accordance with the instructions of the protocasper adjacent motif (PAM), in simple terms PAM serves to control the targeting specification recognized by the Cas9 protein [23].

After undergoing target DNA cutting and producing DSB by the Cas9 protein, the target DNA will undergo DNA repair, by using the non-homologous end merger (NHEJ) and directed repair mechanism (HDR). With an effective method using NHEJ [24]. But the results of this process produce mutations because in the process of repair this will undergo exchange, deletion, and insertion in the target DNA. This makes CRISPR-Cas9 still challenging when applied for clinical applications because it has an off-target effect and causes mutation [25]. Therefore, it needs to be refined, based on Komor's research [26] to minimize the off-target effect, it can be done by optimizing the sequence sequence of gRNA and Cas9. This is like using the Cas9-nickase sequence, the Cytosis-based editor (CBE), the adenine base editor (ABE), and the Prime (PE) editor. With sequences that have been optimized, insertion and deletion result in smaller mutations.

Although the application of CRISPR-Cas9 still has drawbacks to but clinically, based on research [27]. CRISPR-Cas9 is an effective therapeutic method to prevent HPV virus infection, because the therapy can inhibit tumor growth. The first research to apply CRISPR-Cas9 for this application occurred in 2012, by converting gRNA to produce the components needed by prerRNa/tracrRNA to guide the Cas9 protein. And this was successfully done in 2014 by a study [28] by successfully targeting the HPV E6/E7 gene with CRISPR-CAS9 in inhibiting tumor cell growth.

Application of CRISPR-Cas9 in HPV Infection Therapy

CRISPR-Cas9 technology can edit gene sequences specifically and precisely so that it is used as a therapeutic technology in the treatment of various serious diseases, one of which is gene therapy for cervical cancer that causes HPV virus infection. Using CRISPR-CAS9 technology, the target genes E6 and E7 in HPV16 and HPV 18 were inactivated, thereby stopping cell division and apoptosis [29]. Based on research conducted by [30] using CRISPR-Cas9 using sgRNA to target HPV16-E7 DNA in HPV-positive cell lines to be disrupted and induce apoptosis and inhibition of HPV-positive SiHa and Caski cell growth, but not in HPV-negative C33 and HEk293 cells. In addition, it results in increased regulation of pRb tumor-suppressor protein regulation and causes a decrease in the regulation of the E7 protein.

To perform gene therapy using CRISPR-Cas9 in HPV infection, some preparation is required. The first thing is to do cell culture and transfection. There are a variety of cell cultures that can be used for HPV infection listed in the following table.

Table 1. Cell Culture for Cervical Cancer

Genotype	Gen target	Cell/Animal Model	Author/year
CRISPR-Cas9 HPV16	E7	Sel SiHa dan Caski	(Hu et.al., 2014)
CRISPR-Cas9 HPV16	E6 dan E7	Sel SiHa dan C33A	(Zhen et.al., 2014)
CRISPR-Cas9 HPV6/11	E7	Keratinosit manusia	(Liu et.al., 2016)
CRISPR-Cas9 HPV16	E6 dan E7	Sel SiHa dan C33A	(Sun et.al.,2018)
CRISPR-Cas9 HPV16	SIRT1	sel C33A	(Das et.al., 2017)
CRISPR-Cas9	DNAJA4	Sel HaCaT	(Sun et.al.2018)
CRISPR-Cas9 HPV18	E6 dan E7	Sel Hela	(Wang et.al 2018)
CRISPR-Cas9 HPV18	E6	Sel HeLa, HCS-2, SKG dan garis sel abadi manusia 293	(Yoshiba et.al 2019)
CRISPR-Cas9 HPV16/18	E6 dan E7	HeLa, CasKi, HEK293T, garis sel Jurkat dan HeLa FLAG16E7MYC44	(Jubair et.al., 2019)
CRISPR-Cas9 HPV16	SAMHD1	Sel N/Tert-1	(James et.al., 2019)
CRISPR-Cas9 HPV16/18	THZ1	Sel HeLa, SiHa, C33A dan sel ginjal embrio manusia 293T	(Zhing et.al., 2019)
CRISPR-Cas9	CIB1	Garis keratinosit manusia NKc2115 dan garis fibroblast tikus 3T316	(Imahorn et.lal 2020)
CRISPR-Cas9 HPV16	PIM1	Garis sel HNSCC manusia FaDu, SCC-4, SCC-9, SCC-15, CAL 27, Detroit 562, SCC-25, UM-SCC-47 dan UM-SCC-104	(Broutian et.al 2020)
CRISPR-Cas9 HPV16	E6 dan E7	Sel SiHa	(Pirouzfar et.al., 2020)
CRISPR-Cas9 HPV16	WRN	Sel N/Tert-1 dan HPV16	(Ling et.al.,2020)

After cell culture, then plasmid vector construction is carried out to match the HPV virus. To generate a Cas9 expression vector, the promoter part of the cell site is cut and

inserted into the vector cell. Then a CRISPR design was carried out that was adapted to the HPV virus which would later become a target of CRISPR [31]. In its development, HPV16-E7 is the first infection of cervical cancer using CRISPR-Cas9 technology [32] and continues with the development of CRISPR-Cas9 which can accumulate p53 and p21 significantly and reduce the proliferation of E6 and E7 cancer cells [33]. Subsequent studies found that deletions in the E6 and E7 genes can inhibit the proliferation of cancer cells despite having other side effects. Research continues to be developed with confirmation that activating the HPV E6/E7 gene can provide a sensitizer for chemotherapy in cervical cancer, so research continues to be developed by blocking the PD-1 pathway and the HPV E6/E7 gene which provides antitumor effects [34].

The mechanism that occurs in cervical cancer, by CRISPR-Cas9 is targeted to inactivate the oncogene proteins E6 and E7 thereby promoting cell cycle arrest and apoptosis. The research that has been conducted by [35] conducted CRISPR-Cas9 technology using the deletion method. The results showed that this also succeeded in reducing the size of cervical cancer due to the activity of cell apoptosis but did not inhibit the growth of cancer cells.

So based on many studies, CRISPR-Cas9 has potential as a therapeutic agent for the treatment of cervical cancer. Through by means of dispersion, deletion and correction/insertion. And through CRISPR-Cas9 through the knockout function by holding genes without mutations, editing by changing the sequence of genes so that point mutations occur, and inhibitors by reducing gene expression activity so that genes can be modified permanently. Or through editing without modifying the gene permanently [36].

Effectiveness of CRISPR-CAS9 in Therapeutic Medicine

In its development, CRISPR-Cas9 technology is one of the potential therapeutic methods to overcome cervical cancer caused by HPV. This technology allows specific gene editing, by targeting the E6 and E7 oncogenes, so that it can induce the accumulation of p53 and p21, which then leads to cell cycle termination and apoptosis in cancer cells [37]. Studies show that the combination of CRISPR-Cas9 with cisplatin chemotherapy (CDDP) can improve the sensitivity of cancer cells to treatment, thereby increasing the effectiveness of cervical cancer therapy. However, the main challenge in the application of CRISPR-Cas9 is the risk of off-target effects, i.e. accidental mutations in DNA that can cause dangerous side effects [38]. Several strategies have been developed to improve safety, such as the use of Cas9-nickase (the D10 mutant of Cas9) and base editors (Cytosine Base Editors – CBEs and Adenine Base Editors – ABEs), which allow specific DNA modification without causing DNA double-strand damage [39].

Although CRISPR-Cas9 has shown promising results in preclinical research and in vitro trials, its use in cervical cancer therapy is still limited to early-stage clinical trials. One of the first clinical trials using CRISPR-Cas9 in cancer therapy was conducted in non-small cell lung cancer patients in 2020, which opened up its potential use for other cancer therapies, including cervical cancer (Wei et al., 2022). In addition, this technology has also begun to be developed to detect high-sensitivity HPV DNA as well as as a tool in the development of vaccines against HPV [40]. Overall, CRISPR-Cas9 offers great potential as a future cervical cancer therapy, but more research is still needed to ensure its safety and effectiveness before it can be widely used in clinical practice [41].

CONCLUSION

CRISPR-Cas9 is a natural mechanism that occurs in bacteria to fight and kill viral and phage infections. CRISPR-Cas9 is able to perform genome editing by inserting, deletion, and editing the target DNA which has a mutation effect on the editing results.

To be used as a therapeutic agent, CRISPR editing/midification is needed in accordance with viral infection, so that this method is used for therapeutic cervical cancer. In the process, CRISPR-Cas9 will target the Cas9 protein to inactivate the oncogene proteins E6 and E7 in the HPV-16 Virus, thereby promoting cell cycle arrest and apoptosis. Various studies have shown the effectiveness of reducing cell apoptosis and reducing the volume of cancer tissue. So CRISPR-Cas9 should be further developed in order to reduce the global incidence and mortality from cervical cancer.

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