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Antisense Oligonucleotide Protein Therapy to Inhibit Increased Ki67 Expression in Oral Squamous Cell Carcinoma Patients

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1. Abstract

1.1. Background: Oral cavity cancer is one of the most common neoplastic lesions of the head and neck. Relatively recent data suggest that the incidence of neoplastic lesions is quite high. In one study, out of 143 oral cavity tumors, 125 were malignant, of which 115 (92%) were oral squamous cell carcinoma which was ranked sixth in the world. One strategy that can be used is Antisense Oligonucleotide (ASO) which is a protein that hybridizes complementary mRNA sequentially in Watson-Crick base pairing. This protein is therapeutic in malignancy.

1.2. Aims: Provides an overview of the oligonucleotide antisense and its therapeutic application to oral squamous cell carcinoma.

1.3. Discussion: KI-67 is a nucleic protein whose role is closely related to cell proliferation and is widely used as a marker for cancer. Thus, KI-67 is a specific therapeutic target in cancer. The therapy that can inhibit or disable Ki-67 is a promising application in cancer treatment. Antisense Oligonucleotide (ASO) can inhibit KI-67 expression. ASO is a protein sequentially hybridizing complementary mRNA. This protein can be a therapy that blocks the translation process and inhibits protein formation. ASO as a target validator and a therapeutic strategy for malignancies, especially squamous cell carcinoma.

1.4. Conclusion: Antisense oligonucleotide (ASO) can inhibit the expression of KI-67 which causes overproduction by inhibiting the translation of genes in cells.

2. Introduction

Oral cancer is an uncontrolled growth of cells or tissue and is one of the most common neoplastic lesions of the head and neck [15]. Oral cancer is one of head and neck cancers that can be experienced by 3-4% of the population in North America and Europe. Meanwhile, in the United States, head, and neck cancer accounts for 8% of all cancer cases. Head and neck cancer that occurs most often and causes a high mortality rate is oral squamous cell carcinoma. A study stated that, from 143 oral cavity tumors, 125 were malignant with 115 (92%) being oral squamous cell carcinoma which was ranked sixth in the world. Research in the United States stated that there are 21,500 cases with approximately 7,900 deaths due to oral cancer and more than 90% are squamous cell carcinomas [1]. Each year, oral squamous cell carcinoma results in more than about 30,000 new cases. In Indonesia, the prevalence of oral squamous cell carcinoma cases ranges from 3-20% with a mortality rate of up to 3.5%. The prevalence of these cases continued to increase to more than 50% in 2007-2011 [22].

Increased cases of oral squamous cell carcinoma can occur due to people's lifestyle. The current lifestyle of society tends to depend

on a poor diet which can cause infection with other viruses, as well as the consumption of alcohol and tobacco in various forms. Tobacco is one of the risk factors for oral squamous cell carcinoma because it is associated with intra-oral carcinogens that can trigger oral tumorigenesis. These risk factors can cause the death rate from oral squamous cell carcinoma to continue to increase. These cases can decrease if therapy or lifestyle changes are applied [25]. Despite advances in treatment to reduce these cases, the prognosis for patients with oral squamous cell carcinoma is poor because of the local recurrence that can occur from the carcinoma. Several studies state that local recurrence that occurs after treatment in the form of surgery shows as much as 11% to 17% at all stages. Meanwhile, treatment with a combination of radiotherapy and surgery experienced a recurrence of 13-19%. Therefore, it is necessary to have adequate therapy that can be applied to all stages of oral squamous cell carcinoma, such as molecular therapy that can provide better cure rates for some types of neoplasia [6].

Molecular therapy is a therapy that is currently being developed because it is an adequate therapy. One of the molecular therapies currently being developed by dentists and doctors is a therapy that uses a modification of the nucleic acid gene or what is called Antisense Oligonucleotide (ASO) [18]. Antisense Oligonucleotide (ASO) is a chemically modified sequence of nucleic acids. Antisense oligonucleotide (ASO) can be used as a therapy for neoplasia because it can function to inactivate a specific gene that can inhibit the apoptosis of a cell (Frazier and Kendall, 2015). Based on this background, this paper aims to determine the potential of Antisense oligonucleotide (ASO) protein therapy to inhibit the increase in KI67 in patients with oral squamous cell carcinoma.

3. Oral Squamous Cell Carcinoma

Oral Squamous Cell Carcinoma (OSCC) is one of the most common neoplastic lesions of the head and neck [9]. Oral squamous cell carcinoma can affect any part of the anatomy of the mouth but is most found on the tongue and floor of the mouth. This cancer usually arises from pre-existing potentially malignant lesions, and sometimes on a de novo2 basis. Oral squamous cell carcinoma occurs most frequently on the tongue, buccal mucosa, and gingiva, and shows a predilection for men over 50 years of age [20].

The etiology of OSCC is multifactorial and involves both intrinsic and extrinsic factors. Although it is well known that tobacco and alcohol are two major environmental risk factors associated with the development of oral squamous cell carcinoma [11]. The danger of smoking in the mouth tissue is that cigarettes contain about 300 carcinogenic compounds that are converted into reactive metabolites that are able to interact with DNA by the activity of oxidative enzymes, especially polycyclic aromatic hydrocarbons and tobacco. nitrosamine-specific found in tar. Other carcinogens, such as nickel and cadmium, radioactive elements such as carbon-14 and polonium-210, and even pesticide residues used in tobacco growth can also be detected in tobacco and tobacco smoke [8]. Apart from tobacco, alcohol consumption and abuse, and betel nut significantly play a role in the occurrence of OSCC [14]. Substances that are carcinogenic are found in alcoholic beverages. For example, N-nitroso compounds, mycotoxins, urethane, inorganic arsenic, and others. The main metabolite of alcohol is acetaldehyde which causes DNA damage in mammalian cell cultures and interferes with DNA synthesis and repair1. Human papilloma virus (HPV) is also considered as a major cause in the etiology of OSCC because of its ability to retain oral keratinocytes and bring about epithelial cell transformation [23].

4. Pathogenesis of Oral Squamous Cell Carcinoma

Oral Squamous Cell Carcinoma (OSCC) can be found as a result of various molecular events that develop from the combined effects of an individual's genetic predisposition and exposure to environmental carcinogens, such as tobacco, alcohol, chemical carcinogens, ultraviolet or ionizing radiation and microorganisms. Chronic exposure to carcinogens can damage individual genes as well as larger portions of genetic material, such as chromosomes, which can activate mutations or amplification of oncogenes that increase cell survival and proliferation. Mutations include general DNA hypomethylation, hyper or hypomethylation of certain genes such as D-cyclin, and changes in chromatin [17].

5. Oral Squamous Cell Carcinoma Treatment

All major surgical treatment modalities, radiation therapy and chemotherapy (CT) are used to treat OSCC, either alone or in combination. In general, single modalities are more commonly used in the early stages of OSCC (Stages I & II) and carcinoma-in situ (CIS), whereas patients with more advanced stages (Stages III & IV) are treated with combination therapy. At an advanced stage, given treatment such as surgery and neck dissection and / or radiation or surgery which is then performed neck dissection, radiation and / or chemotherapy or surgery which is then performed neck dissection, radiation and / or targeted therapy [12]. OSCC patients undergo surgery with other combined therapies. Patients in clinical stage I-II are treated with induction chemotherapy before surgery and surgery, while patients in stage III-IV undergo preoperative chemotherapy, surgery, and postoperative radiotherapy or chemotherapy. TFP regimens are used for preoperative and postoperative adjuvant chemotherapy: docetaxel at 60 mg / m2, day 1; cisplatin at 60 mg / m2, day 2 to day 4; and 5-fluorouracil at 750 mg / m2, days 2 to 6 [24].

6. KI67 Expression

Ki67 is a molecular biomarker protein for cell proliferation and is associated with tumor invasion, differentiation, metastasis and prognosis. Ki67 is a prognostic and predictive indicator used for biopsy assessment of cancer patients. Clinically, Ki67 has been shown to correlate with metastasis and the clinical stage of tumors [16]. Ki67 is a protein present in the nucleus, whose function is

related to cell proliferation. Some literature states that high levels of Ki67 expression are associated with a poor prognosis [5].

Ki67 is a marker of cell proliferation that is useful for determining growth fraction in tumor cells during the active phase of the cell cycle. Cells normally undergo mitotic division in a cycle called the cell cycle, which functions to produce new cells that are useful for regeneration and for repairing damage, this sequence is regulated by DNA sequences in each cell. Cells have genes that regulate cell proliferation called protooncogenes such as the Ki67 gene and genes that function to regulate the termination or inhibition of cell proliferation called gene suppressors such as p-53. These genes function as control, if these genes undergo mutations, the related proteins are not formed properly and cell division occurs which should not occur. Mutation of the Ki67 gene in the mitotic phase causes uncontrolled cell division resulting in the movement of cells undergoing division and encourages tumor cells to pass through the damaged basement membrane or lysis and enter the circulation (bloodstream). The tumor cells that form these clots will spread haematogenously and eventually enter the blood vessels and can directly invade the blood vessels through the vena cava so that they can be detected in the blood [16]. Antisense oligonucleotides and antibodies to pKi67 have been shown to inhibit cell cycle development. The Ki67 protein is characterized at the molecular level and is extensively used as a prognostic and predictive marker for the diagnosis and treatment of cancer. A growing body of evidence suggests that Ki67 may be an effective target in cancer therapy [5, 16].

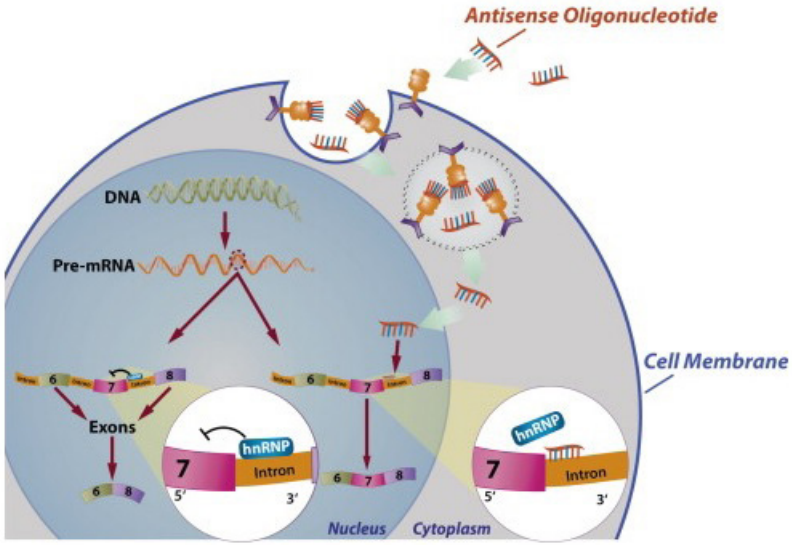

Figure: Antisense Oligonucleotide Protein Mechanism

7. Antisense Oligonucleotide Protein (ASO)

Antisense Oligonucleotide (ASO) is a short synthetic strand of deoxyribonucleotide that functions to hybridize with complementary mRNA via Watson-Crick base pairs. mRNA in duplex RNA-DNA substrates for Cellular Ribonuclease H [RNase H] is an enzyme that destroys RNA. RNase H will cut the duplicate area of RNA-DNA from mRNA, thus triggering a blockade in the transmission of genetic information from DNA to protein. Antisense oligonucleotide (ASO) has been used to modify gene-specific expression. Antisense oligonucleotide (ASO) is not only useful in loss-of-gene function studies and target validation, but also as a novel therapeutic strategy to treat any disease associated with unregulated gene expression. Antisense oligonucleotide (ASO) can also manipulate alternative splicing, so that it can be used to modulate the ratio of different splicing variants or defects in the correct splicing [2].

Antisense oligonucleotide (ASO) is a short nucleic acid polymer designed to hybridize and modulate specific cellular RNA function. Antisense oligonucleotide (ASO) is used in many basic studies and as a therapy to target RNA in the cleavage and degradation of H1-mediated Rnase19. Antisense oligonucleotide (ASO) as synthetic genetic material that interacts with natural genetic material and can modulate this genetic material in a systematic way. Antisense oligonucleotide (ASO) is a molecular treatment for modulating gene function that was first recognized in the late 1970s. This therapy involves blocking translation, thereby inhibiting protein formation. Recently, antisense technology has been redeveloped and has generated great enthusiasm for research. Antisense oligonucleotide (ASO) has been shown to play an important role in gene functionalization and target validation and is also a new therapeutic strategy for various diseases such as genetic disorders, cancer and infectious diseases [3].

Antisense Oligonucleotide (ASO) is obtained from cell endocytosis, then hybridization occurs with target mRNA resulting in the formation of antisense oligonucleotide-mRNA heteroduplex which modulates either H RNAse activation or steric binding of ribosomal subunit barriers. Both of these mechanisms result in se-

lective degradation of bound mRNA and target protein knockout. RNase H-dependent oligonucleotides can induce mRNA degradation when targeted to any mRNA. However, steric-blocker oligonucleotides prevented progression of splicing only when targeted to the 5 'or AUG initiation codon area. Another mechanism that can be used by antisense oligonucleotide (ASO) is to enter the nucleus directly and change mRNA maturation, splicing activation, inhibition of 5'-cap formation, translation capture and activation of RNA double strands [3].

8. Discussion

In tumor biology, proliferation is a highly visible feature of the presence of cancer and acts as an important determinant of cancer prognosis. Cell proliferation is also seen as more aggressive clinically. No exception to the oral squamous cell carcinoma (OSCC) which is the most common cancer of the tongue and floor of the mouth. This cancer usually arises from pre-existing potentially malignant lesions. In OSCC, there is activation of gene mutations that can increase cell proliferation activity [24]. Cell proliferation is an important adjunct to histologic classification of tumors and has potential relevance as an indicator of treatment response and recurrence. Many studies have reported that abnormal cell proliferation appears to be a precursor and may be a predictor of tumorigenesis [7].

Ki67 antigen is a specific marker of proliferating cells. A number of studies have demonstrated a highly significant correlation between Ki67 and malignancy, and marked variation in different tumor rates, suggesting that Ki67 is useful in tumor diagnosis and prognosis. Ki67 immunostain was identified in all OSCC cases and correlated with the degree of dysplasia and degree of OSCC differentiation and showed high values and intensity at the onset of tumor invasion [7, 4]. In OSCC, increased Ki67 correlates with poor survival rates, high levels of malignancy and histological values at the onset of invasive OSCC [9]. Ki67 positive cells in OSCC are located peripherally to the tumor from the center, where mitosis is common. The nuclear expression of the Ki67 antibody is calculated based on the epithelial / strata layer as the basal layer, with the positive nucleus just above the basement membrane i.e. the parabasal layer, the positive nucleus in two layers above the basement membrane and next to the basement layer, and the suprabasal layer, with nuclear positivity in the layer or in the layer above the parabasal layer [4].

Through research in the field of molecular biology, it is known that organisms are regulated by their genes and their expressions. The regulation of gene expression is a complex process and involves a variety of factors. Abnormalities in the base arrangement of genes and their expression can cause a number of diseases, including cancers that have high KI67 levels. Here efforts to inhibit gene expression are considered to be an alternative in cancer.

The molecular activities that produce Antisense Oligonucleotide activity (ASO) are divided into three phases, namely pre-hybridization, hybridization, and posthybridization. In the pre-hydridation phase, Antisense Oligonucleotide (ASO) must enter the cell, distribute itself into the cell, and reach an effective concentration in the target portion of the RNA. It then sorts the cellular nucleic acid sequence sites to hybridize to the place of origin. The concentration of the cognitive portion was significantly less relative to total cellular RNA and more relative to the conditions used in the in vitro hybridization assay. Hybridization to the cognitive part of the cell is a much more complex process than in a test tube and must involve interactions with proteins, such as Ago2, or other cellular components that facilitate hybridization. Once the antisense oligonucleotide (ASO) binds to its site of origin, depending on the chemical structure of the antisense oligonucleotide (ASO), various activities can be induced that alter the target RNA to achieve the desired pharmacological results [21].

Antisense oligonucleotide (ASO) is captured by cell endocytosis, hybridization with target mRNA results in the formation of antisense oligonucleotide-mRNA heteroduplex in most of the time for H RNAse activation or sterichindrance of ribosomal subunit bonds. Both of these mechanisms result in selective degradation of mRNA-bound and ultimately target protein [3, 4]. Antisense oligonucleotide (ASO) is made synthetically with a base sequence that is complementary to the mRNA target strand so that the hybridization of the antisense oligonucleotide with the mRNA strand results in the ribosome complex unable to read the message carried by mRNA. Inhibition of gene expression occurs at the transcription stage through the formation of a twisted three-strand or at the translation stage through mRNA hybridization with antisense. The first inhibitory process is by inhibiting the binding of the ribosome complex with mRNA at the 5 '(5' cap site) end. In the translation stage, the binding of antisense to the adjacent end of the intron and exon results in inhibition of the splicing process which indirectly inhibits gene expression.

The mechanism of action of antisense oligonucleotides (ASO) is by the entry of antisense oligonucleotides (ASO) into cells through high- and low-binding plasma protein receptors on the cell surface, resulting in Antisense Oligonucleotide compartments (ASO) into lysosomes and endosomes. antisense oligonucleotide (ASO) is released from the vesicles into the cytoplasm where it can freely move in and out of the nucleus. After entering the nucleus, the antisense oligonucleotide (ASO) binds directly to the mRNA structure and prevents the formation of a 5'-mRNA cap, which will modulate the alternative splicing process. Here, the polyadenylation location will be detected and RNaseH1 will induce cleavage [3].

Antisense Oligonucleotide (ASO) in the cytoplasm can bind directly to the target mRNA and block ribosomal subunits from attaching along the mRNA transcript during the translation process. Antisense Oligonucleotides (ASO) can also be designed to direct-

ly bind to microRNA (miRNA) and natural antisense transcripts (NATs), thereby preventing miRNA and NAT from inhibiting their own specific target mRNA. So that it can modulate gene upregulation of miRNA and NAT targets [19].

Inhibition of gene expression (antisense) in question is Antisense Oligonucleotide (ASO). Ki67-antisense oligonucleotide can inhibit tumor growth, blocking Ki67 either by micro-injection of antibodies or through the use of antisense oligonucleotides by inhibiting cell proliferation. In particular, the Antisense oligonucleotide (ASO) in Ki67 has been shown to inhibit cell cycle development17. Antisense oligonucleotide (ASO) was injected into the OSCC mass daily. Tumor size, body weight and duration of survival were assessed on a daily basis. The specimens of the mass were primarily used for immunohistochemical staining to analyze the expression of proliferating cell nuclear antigen (PCNA) and matrix metalloproteinase-2 (MMP-2) [13].

9. Conclusion

Antisense oligonucleotide (ASO) can inhibit the expression of KI67 which causes the proliferation and growth of malignant cells by inhibiting gene expression at the transcription and gene translation stages.

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