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Review Paper

FIGHTER MOLECULES AGAINIST TO CANCER – PEPTIDE VACCINES

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ABSTRACT

Cancer is one of the biggest disease of our age. Chance of survival decreases as the diagnosis prolongs. Being an insidious disease makes it harder to determine and diagnose early for cancer. There are lots of studies about fighting this disease. In recent years, vaccine studies have gotten important to prevent cancer.
Although cancer vaccine studies have a long history, studies which use peptide vaccines for cancer are applied about last 20 years. Peptide vaccines are created by using peptide sequences which generate the disease as template. The aim is to destroy the tumour antigens by T lymphocytes which are activated by the immune system using peptide sequences.
Keywords: Cancer, peptide vaccine, carrier molecules, immunotherapy.

1. INTRODUCTION

The uncontrolled multiplication of mutated cells via various effects causes cancer. These cells continuously increase the places where they occupy, distant tissues and organs and prevent to do one's part for the tissue and organ. As a result of this death occurs [1].

Cancer is one of the health problems which is frequent and has a high death rate. According to World Health Organisations (WHO)' reports, it is believed that number of cancer patient will be over 25 million in 2025. This is why, studies about cancer diagnosis and treatment are rapidly increasing and treatments with no or few side effects are being studied [2]. According to 2008 data, 12.7 million cancer patients were recorded and 7.6 million of them lost their lives [3].

The most frequent cancer types for men in Turkey are; lung, prostate, colon, rectum, stomach and pancreas cancers. On the other hand the most frequent cancer types for women are; breast, lung, colon, rectum, cervix, ovarian, stomach and pancreas cancers [4].

As shown in figure 1, while proliferation of normal cells ends with cells touch each other, cancer cells show no behaviour like this. Cancer cells evolve to escape from immune system and provide blood circulation for themselves to survive in the body [5].

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Diffusion of cancer cells from where they are to whole body is called metastasis. Metastasis, causes the organs to lose function which results with the death of the patient. It is very important to diagnose cancer early in order to prevent the metastasis and diffusion of cancer cells to whole body [4].

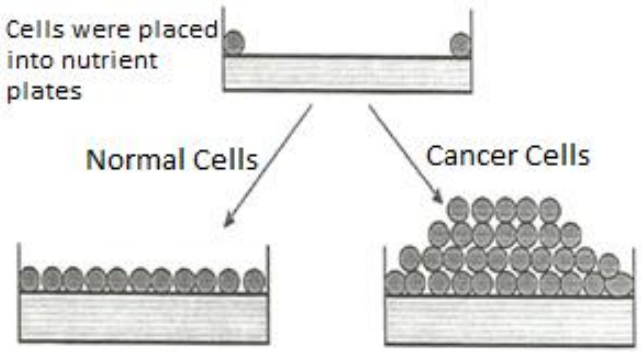

Figure 1. The difference between normal and cancer cells proliferation [5].

2. CANCER IMMUNOTHERAPY

Studies have focused on vaccine development for the cancer treatment. Vaccines ensure protection by stimulating humoral and cellular immunity. The peptide sequences that are used in these vaccines are obtained by chemically or by isolation. Obtaining the peptides chemically has advantageous because it does not use viable pathogen which makes it safe and this method gives the peptide a higher chemical resistance. However, due to their small sizes, it is necessary to connect peptides to carrier molecules so they can show high immunity. Peptide vaccines; use the amino acid series of an antigenic peptide epitope of a specific disease which are synthesised in various methods to target cancer cells [6].

In recent years, effects of immunotherapy are steadily increasing in cancer. Normally, immune system has the ability of detect the cells that breed abnormally. In some cases, various cancer types can keep breeding by deactivating the defence mechanism. There are plenty of studies done to activate immunotherapy by using monoclonal antibodies, interferons, interleukins and cancer vaccines. In the future, immunotherapy can actively be used in cancer treatment [7].

As shown in figure 2, active and passive immunotherapy of cancer therapy is to activate the immune system against cancer cells. T cells have the ability to sense the signals of various pathogens and cancer. It gets activated when it senses the tumour related antigens. Activated T cells can recognize the antigens on the tumour. The biggest challenge of cancer treatment is determination of the tumour antigens. MHC class 1-limited T cells are capable of identifying tumour cells. However, cancer cells can repress the MHC 1 molecules and escape from the immune system [9]. Vaccine studies continue to prevent this situation.

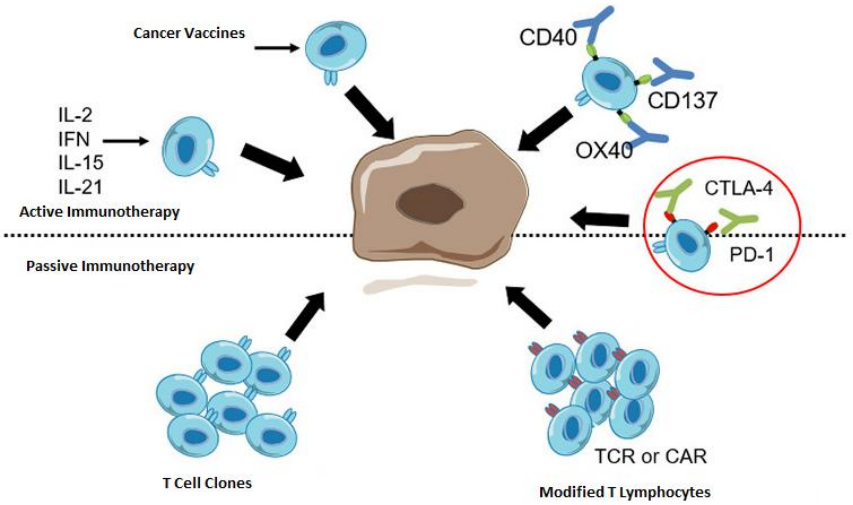

Figure 2. Def ence mechanisms against to cancer cells [8].

3. CANCER VACCINES

The key matter in cancer treatment is to activate the immune system [5]. The aim of the treatment is to destroy the cancer cells without harming the healthy cells. Immune system cannot distinguish between cancer cells and healthy cells. This is why the immune system cannot recognize the cancer cells as enemies and generate immune response. Studies are focused on this when developing cancer vaccines. The aim of the cancer vaccines that are designed is to help the immune system to recognize the cancer cells and produce a response to them [10].

Cancer vaccines were tested for various cancer types. It is determined that production of growth factor is increased in cancer patients. Peptide, protein or DNA vaccines target these growth factors by using various adjuvants.

- In production of cancer vaccines, various antigen groups were studied. Within these groups TRP2 protein was targeted for melanoma. TRP2 is tissue specific differentiation antigen in melanoma tumour cells [11].
- The other antigen group studied increased a lot in tumour cells and promoted the formation of tumour. One of these antigens is epidermal growth factor receptor 2 (HER2) which was associated with breast cancer [12].
- Studies about epidermal growth factor receptor for colon cancer were performed [13].

There are some disadvantages of antibodies used in vaccine studies. Their large size and difficulty of uptake into cell are some of the biggest of these disadvantages. These situations inhibit the movement to tumour area. Using peptides instead of antibodies can take away these problems [14]. Studies that use peptides in cancer treatment on going [15]. Even though there is no cancer peptide vaccine in produced yet, studies on animals continue rapidly [5].

4. BIOTECHNOLOGICALLY PEPTIDE VACCINES

Peptide vaccines are obtained from protein antigen sequences which are synthesized from amino acids that are collected on a single molecule, formed a supermolecule complex or just a mechanical mixture. These vaccines are recognized by the immune system and cause an immune

response [16]. This immune response may include the other T or B cells or can form complexes of both pathways [17].

Protein molecule particles show B and/or T epitope activity which are the main components of peptide vaccines. These epitopes adjust the course and specificity of the immune response. Some vaccines may also contain some components which are specific to the individual or supermolecule complexes. These individual components can activate the stages of immune response to peptides by specific or non-specific ways [18]. The increase in the chemical stability of peptides is due to their bonding with carrier molecules. In this case, carrier molecule called adjuvant and plays role in the increase of immune response [19].

After the type and number of the amino acid is determined from the immunogenic components of pathogens like virus, bacterium and helminth, chemical synthesis of these components is performed in laboratory conditions. Obtained peptides can be used as a vaccine by bonding it to a carrier. After peptide-polymer molecule is injected to the body, immune response against to the peptides initiates and antibodies are produced [20].

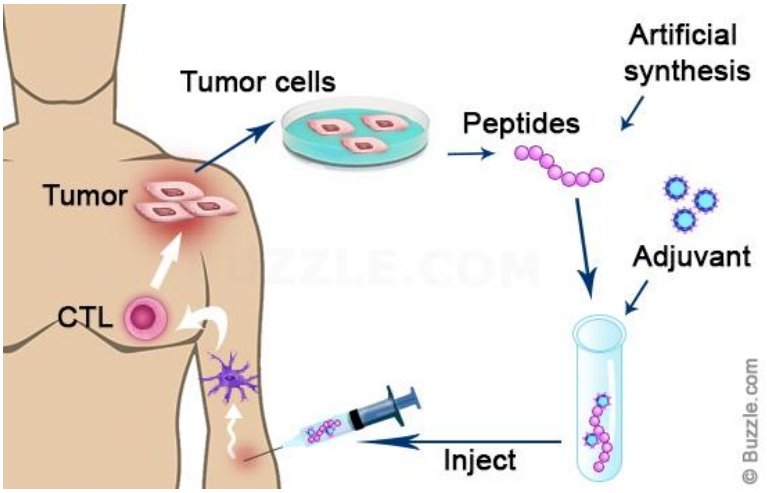

Figure 3. Action mechanism of the peptide vaccine [21]

It is necessary to determine the antigenic peptide sequence that can stimulate the immune system, in order for it to be used as vaccine. The determined peptide sequences are injected into the patient by combining with the adjuvants as shown in figure 3. The immune system is intended to act and destroy tumor movements. Advantages of using peptide vaccines instead of classical vaccines are:

- No mutation risk.
- Relatively inexpensive and safe production technologies.
- Various antigenic sequences can be applied.
- Peptide vaccines have the ability of stimulate immunity [22].

There are some challenges of using peptides as vaccine. These challenges arose because of the structural features of peptides. High instability of peptides is caused by fast emulsion through the plasma and weak transport between membranes. In order for peptides to show the desired effect the amount of peptides reaching to target area must be over a certain amount. Peptidase enzyme which exists in the bloodstream and in tissues may break the peptide in to pieces before reaching the target area. Duration and activity of immunity from peptides may be insufficient when they are introduced to the body unaccompanied. Peptides are rapidly catabolized in the body and

cannot stimulate the immune system sufficiently. This is why multiple vaccination are necessary [22]. This situation is one of the obstacles which must be overcame in vaccine and medicine studies [23].

An adjuvant is a substance that is used to increase efficiency of the vaccine and to stimulate the immune system more effectively by increasing its' residence time in the body. Especially peptides are connected to adjuvants and can increase its efficiency [22]. Adjuvants help the formation of a strong and long-term specific immune response [24]. Peptide vaccine studies for cancer treatment or viral infections are rapidly on going [25].

5. STUDIES ABOUT PEPTIDE VACCINES AGAINIST TO CANCER

5.1. Skin Cancer and Peptide Vaccine Applications

Skin cancer is a type of cancer that becomes more frequent with every passing year. An estimate of 3.5 million of new skin cancer patients annually has been made [26]. Skin cancer exhausts the patient financially and spiritually [27]. Skin cancer occurs mostly on head and neck region; however, it may occur on the whole body [28].

Janice et al. tested the activity of melanoma-related antigen-derived peptide (MART-1) in their peptide vaccine studies [29]. It was studied that whether or not it caused a cellular immune response under *in vivo* conditions of Phase-1 active vaccination protocol. In this study, the application of MART turned to emulsion in IFA was investigated. MART-1 was synthesized for HPLC purification and for *in vitro* analyses by solid phase method. The application of this method on melanoma patients was permitted to see the immunological evaluation for cancer treatment. Some of the lesions have shown reduction in three patients. *In vivo* application of melanoma-related antigen-derived peptide can increase the CTL activity against to epitopes expressed by melanoma cells [29].

In a study done by Kızılbey et al. NY-ESO-1 $_{155\text{-}163}$ (Q-L-S-L-L-M-W-I-T) and MAGE-3$_{121\text{-}134}$ (L-L-K-Y-R-A-R-P-V-T-K-A-E) sequenced synthetic peptides belonging to melanoma cancer were synthesized in microwave supported solid phase peptide synthesis method [30]. Peptide-polymer vaccine prototypes were formed by bonding these studied peptides to various polymeric adjuvants P(VP-co-AA)covalently in the presence of crosslinking agents. Toxic effects of these formed prototypes on MCF-7 cells were tested using MTT technique and the biocompatible samples of these peptide-polymer vaccine prototypes were injected in to the Balb/c mice and antibody responses were measured by Indirect Enzyme-Linked Immuno Sorbent Assay (ELISA) technique. It was seen that the obtained prototypes caused a specific immune response by activating the immune system of Balb/c in an ideal way. This study is an important step taken to develop a protector to melanoma cancer and to obtain specific antibodies that will used in tumour diagnosis [30].

5.2. Head-Neck Cancer

Head-neck cancer is a type of cancer that causes physical impairments that effect swallowing and talking. The loss of confidence can be seen in patients who have deformity caused by head-neck cancer because of increasing emphasis on physical appearance in our day [31]. The incidence of head-neck cancer in men is 4.5 times greater than women [32].

P53 accumulation in flat carcinoma cells of head and neck creates targetable tumour antigen. Patrick et al [33] tested phase-1 clinical study of adjuvant dendritic cell-based vaccination against to p53 in their study. The study was conducted on 16 patients. 3 types modified peptide were used.

Vaccination was tolerated well in all patients. Itching on the skin occurred about the injection area. No diversity was seen in 3 vaccines applied. All patients were checked up clinically in every 3 months and PET/BT scannings were tracked for 3 years.

It should not be overlooked that the patients were treated surgically or with chemo-radiation before the vaccination. The action mechanism of the vaccines is not known yet. It may be an advantage to start a tumour specific vaccine study before radiotherapy. The absence of serious side effect in this study shows that number of vaccination can be increased allowing the positive immune response against to p53 peptides to increase. The clinical results of the p53 vaccinations in head and neck cancer patients are promising [33].

5.3. Colon Cancer

Colon cancer is one of the most common cancers and causes 500.000 death in a year [34]. Today, peptides play an important role in diagnosis of colon cancer [35].

In the study of Inoda et al., vaccine prepared with peptides was tried on colon cancer. They used three peptides together and saw that it has an effect on HLA-A24 [36]. Hazema et al. prepared a peptide mixture, applied it to the cancer patients and obtained positive results [37].

Yangde Zhang et al. achieved a successful scanning protocol by using CO15 peptides against colon cancer [38]. Scanning protocol was performed on colon cancer cells and normal human intestine epithelial cells. Normal human intestine cells were used as control group. It was observed that CP15 peptides can have therapeutic effect like an antitumor medicine. In this study, colon cancer tissue specific peptides were chosen. According to the results of the study, CP15 peptide can be used in the diagnosis and treatment of colon cancer [38].

In the *in vivo* studies done by Arap et al. it was seen that peptides destroyed tumours. Nevertheless, tumour cells smaller than 1 mm^3 can uptake nutrients from neighbouring healthy blood vessels which allow these cells to get away from the targeting [39].

5.4. Breast Cancer

Breast cancer is 30% of cancers that are seen in women. Number of breast cancer patients is rapidly increasing in the whole world [40].

HER2/neu is one of the investigated tumour related antigens from breast cancer. In their study, George et. Al. researched the HER2/neu and found that specific CTLs' for ovarian and breast cancer recognize the 9 amino acid peptide. These peptides express HER2/neu tumours.

According to the data obtained from the study, GP2 peptide expression is more efficient in ovarian cancer. GP2 peptide shows cytotoxicity towards tumour cells. The peptides used formed the CTL response. GP2 peptide is more efficient than GP1 peptide. The result of this study showed that GP2 peptide may be useful in peptide vaccine studies. It is believed that if these peptide vaccines will be developed, they can be used for breast and ovarian cancer in the future [41].

6. DISCUSSION

It can be seen from the performed studies that the obtained results are promising even though peptide vaccines are still in the experimental stage. Our group have performed various studies about this topic before [42], [43], [44], [45]. Throughout these study, it was seen that peptides tested in clinical applications can be effective in the fight against cancer. We believe that successful results about this matter will be obtained in Turkey and the world in the future.

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