APPLICATION OF 3D MODEL OF CANCER CELLS IN RESEARCH ON THE EFFECTIVENESS OF BNCT IN THE TREATMENT OF MELANOMA*

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Melanoma is one of highly aggressive kinds of skin cancer with a metastatic potential. Currently, melanoma treatment has been improved by a combination of therapies with targeted kinase inhibitors and immunotherapies, with radiation therapy (RT) reserved to the most advanced melanoma stage. Among different RT options, the Boron Neutron Capture Therapy (BNCT) is a modality which selectively destroys tumor cells leaving healthy ones almost intact. Its therapeutic effect depends mainly on the boron concentration in a cancer. Therefore, there is a need to find a ligand which transfers sufficient amount of boron to cancer cells and study their potential therapeutic activity in preclinical studies. Spheroids, which are 3D cell models are proposed to test nucleic acids as a boron carrier for BNCT to mimic 3D structure and microenvironment of cancer.

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1. Introduction

Melanoma is one of the most aggressive types of skin cancer and its occurrence continues to rise worldwide. Global melanoma incidence is estimated to 1.7% of all new diagnosed melanomas and 0.7% of all cancer deaths are resulted by cutaneous melanoma annually [1, 2]. Melanoma is caused by neoplastic transformation of melanocytes, highly specialized skin cells producing pigment — melanin (dark eumelanin and reddish pheomelanin). Despite over 95% of melanomas are developed in the skin, they can also be found in other parts of a human body like eyes, mucous membranes of the nose, mouth, or less often in vagina and anus. In cancer development, two factors play an important role: environmental exposure

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(UV light) and genetic predispositions [2, 3]. Different mutations are responsible for melanoma transformation among them, two are commonly found in melanomas: $BRAF^{V600E}$ and $NRAS^{Q61}$, in about 40% and 20% of cases respectively [1]. Melanoma treatment is determined by many factors, but the prime and most important are the size and stage of the cancer. There are several therapy modality options: surgery, chemotherapy including biological and targeted therapy, and radiotherapy, which is currently used in a terminal stage of this disease. There is a need to develop and introduce alternative strategies to treat melanoma in late stages successfully. In such a case, the Boron Neutron Capture Therapy (BNCT) could be the best option to save patient life or extend a surviving rate [4].

2. Boron neutron capture therapy

Selective accumulation of ^{10}B in tumor cells and irradiation with thermal neutrons destroys only the cancer without serious damage to adjacent normal cells. The main limitation of this therapy is the lack of a selective and efficient boron carrier to transfer a boron atom to a cancer cell directly and selectively. Currently used boron carriers are not perfect and there is a need to find a better one to increase the success rate of the BNCT therapy and decrease side effects in healthy tissues of a patient [5]. In the BNCT therapy, a non-radioactive boron isotope is injected into patient's body. This isotope is uptaken mainly by cancer cells, which is associated with increased metabolism of these cells. Next, the patient is irradiated by neutron beam. Thanks to high cross section for thermal neutron capture on boron, the dose is deposited mainly in the cancer cells. The products of the neutron capture on ^{10}B reaction have a high linear energy transfer and a low range ($< 10\ \mu$m), hence the energy deposition is limited to the diameter of a single cell (Fig. 1). Therefore, it is possible to selectively destroy only tumor cells while leaving the normal ones intact within a body [6, 7].

The important limitation which affects the efficiency of BNCT is obtaining a sufficient number of boron atoms deposited within each cancer cell. Therefore, the boron delivery system is crucial for the BNCT therapy. Main objective of current research is finding a suitable model to test a boron carrier which could increase its uptake only by tumor cells. Spheroids, which are 3D cell culture models are proposed for such testing allowing to perform this kind of research apart from animal tests.

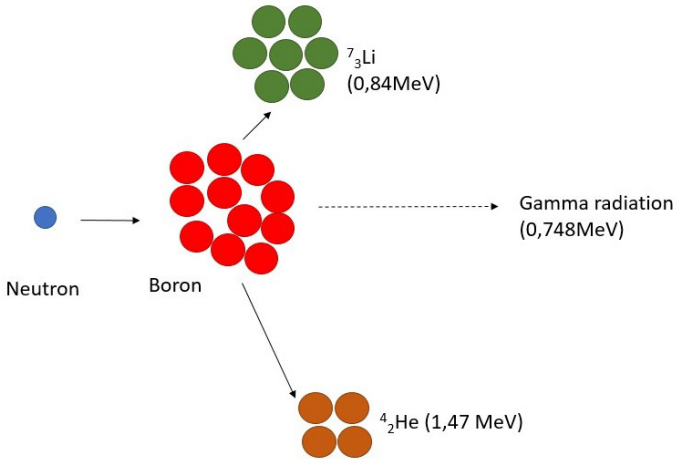

Fig. 1. Schematic representation of the reaction of neutron capture on ^{10}B. Figure adopted from [6].

3. Boron carriers and conveyors

From biological and clinical point of view, the best carrier should be non-toxic for cells. A good proportion of its accumulation in tumor and healthy cells is also very important ($> 3 : 1$). Such a carrier should be quickly removed from patient's body, on the other hand, should also persist in cancer cells during irradiation with a proper concentration ($\sim$ 20–50 μg ^{10}B/g) [8]. Currently the BNCT therapy employs mostly two boron carriers: sodium mercaptoundecahydro-closo-dodecaborate (BSH, $Na_2^{10}B_{12}H_{11}SH$) and boronophenylalanine (BPA). Both of them have, however, their limitations, they accumulate inside cells with low efficiency or stuck outside them. Therefore, there is a need to develop new carriers to improve a system which could provide the sufficient boron inner concentration inside a tumor cell. To accomplish this purpose, several new approaches have been established. Over the last years boron delivery system development has taken two directions: boron carriers and conveyors. Boron carriers include carboranes, amino acids, growth factors or monoclonal antibodies [4, 8, 9] (Fig. 2).

On the other hand, liposomes are very promising conveyors to transfer boron carriers into a cell [8]. Due to their structure, hydrophilic and hydrophobic molecules such as proteins, RNA, DNA, enzymes, vaccines and imaging agents can be carried by them. Two types of liposomes are in use: one to encapsulate radioactive agent inside, the other to incorporate active lipophilic substance to bilayer of liposomes and utilize them to transport these compounds to different places in the patient's body. They have numerous unique properties as drug delivery system including biocompatibil-

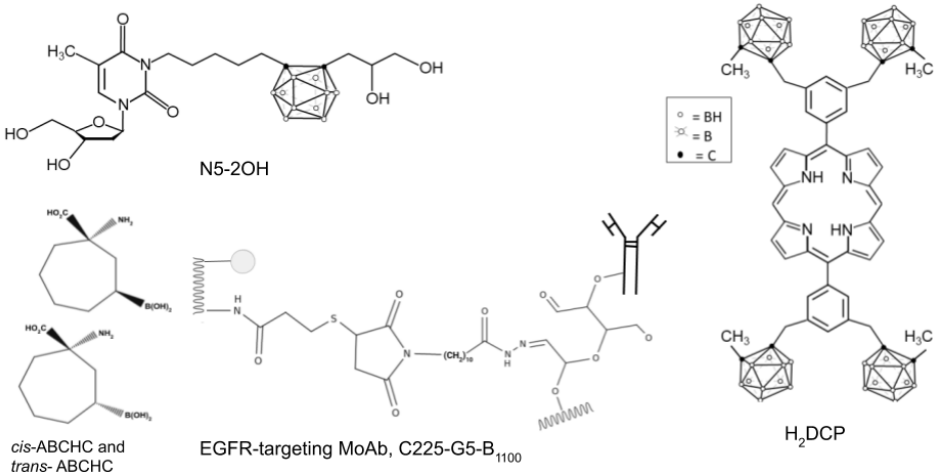

Fig. 2. Different kind of boron carriers. (1) N5-2OH (3-[5-{2-(2,3-dihydroxyprop-1-yl)-o-carboran-1-yl}pentan-1-yl] thymidine) is a carboranyl thymidine analogue (CAT). (2) *cis*-ABCHC and *trans*-ABCHC (1-amino-3-borono-cycloheptanecarboxylic acid). (3) C225-G5-B_{1000} is a heavily boronated form of the monoclonal antibody cetuximab that specifically targets the human epidermal growth factor receptor (EGFR). (4) H_2-DCP (di [3,5-(*nido*-carboranylphenyl) tetra-benzoporphyrin]) is one of a group of carboranyl porphyrins containing multiple carborane clusters. Figure adopted from [8].

ity, biodegradability, low toxicity and immunogenicity, ability to carry large drug cargo. It is possible to modify the surface of liposomes with ligands for site-specific delivery of drugs to determined cell types. Such ligands may be proteins, peptides, antibodies or carbohydrates [10, 11]. Peacock *et al.*, in [12] synthesized a new cholesterol–carborane conjugate as a potential targeting agent for BNCT. This compound was carried by liposomes, resulted in adequate cellular uptake. It was proven that cellular uptake depends on concentration of cholesterol–carborane conjugate in the media and also on confluence of the cells. Nonconfluent cells uptake boron better, which indicates that active cell growth has influence on the uptake of this compound [12].

Testing of boron carriers requires an appropriate research model. Due to the future use of carriers in the treatment of cancer, the model should be as similar to the tumor as possible. Spheroids have a lot of features allowing them to mimic structure and microenvironment of the cancer. Structure of spheroids, as in tumors, consist of different cell layers. The external layer is generated by cells with high proliferation rates, which have unlimited access to nutrients and oxygen. In the center of spheroid, a necrotic zone is

observed. It is caused by conditions that prevail inside the spheroid (hypoxia and lack of nutrients). Another similarity is a possibility of making an intercellular membrane contact by desmosomes, gap junction and extracellular matrix (ECM) production. This kind of cell–cell signaling and presence of ECM makes the organization and structure of spheroid stable and more resistive to radiation [13, 14]. Spheroids have a similar growth pattern to tumors. Firstly, a volume of spheroids increases exponentially then, when the diameter reaches 200–500 μm it enters a plateau state. Molecular studies have shown alike gene expression pattern in spheroids and tumors. There are mostly genes that are responsible for cancer progression, invasion and metastatic as chemokines, cell-adhesion molecules and pro-angiogenic factors [13]. All these attributes make them a good model to test effectiveness of a boron carrier.

4. Melanoma and boron nucleic acid carrier

About 40% of all melanomas have mutation in the BRAF gene which produces a protein — kinase B, Rapidly Accelerating Fibrosarcoma. This protein played significant a role in cells growth and regulating proliferation. Therefore, it is very important in healthy cells, and the mutation in BRAF gene causes uncontrolled proliferation of cells and tumor development [1], see Fig. 3.

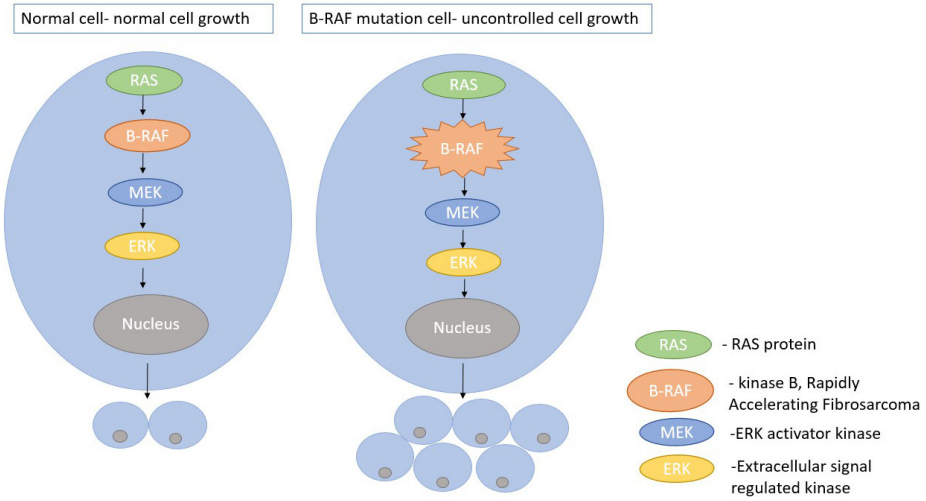

Fig. 3. Formation of tumor. Scheme of differences in growth of a healthy and tumor cell. In a healthy cell the BRAF protein is one of the enzymes which is involved in transmission of the cell growth and division signal. A mutation in this gene disrupts function of BRAF protein which causes uncontrolled cell growth and division. Figure adopted from [15].

Leśnikowski *et al.* have focused their research on nucleic acids and oligonucleotides application as potential boron carriers for BNCT [16–18]. In their research, a few important features of this carriers such as low cytotoxicity, increased lipophilicity, and formation of stable heteroduplexes with complementary RNA strains were proven. In another approach, impact on various boron clusters incorporated into the structure of nucleic acid at specific locations was examined. They showed also that oligonucleotides with boron clusters may have an additional role like silencing activity, redox active labels for electrochemical detection of DNA [16]. Boron clusters were tested to modify siRNA directed toward the gene encoding BACE1. This gene produces protein contributing to the formation of peptides, which is identified in amyloid plaques of patient with Alzheimer's disease. According to the amyloid cascade hypothesis, inhibition of production protein encoding by BACE1 may be one of the approach for Alzheimer's disease treatment. It could be possible by blocking the expression of BACE1 by appropriate siRNA [18].

In the current project, boron carrier based on nucleic acid will be used. A working hypothesis assumes that sequences with mutation in BRAF gene [19, 20] could serve as templates to synthesize oligonucleotide containing a boron cluster [18]. This oligomer would be preferentially combined with melanomas DNA, due to the presence of the same mutation. It is planned to use the synthetic RNA oligonucleotides which are complementary to particular, selected several BRAF mutations and conjugate them with a boron cluster. These boron-loaded antisense RNA-oligonucleotides will be encapsulated in liposomes and tested with utilized spheroids. Examination of viability and DNA fragmentation in spheroids after neutron irradiation is planned. Evaluation of BNCT effectiveness using this type of boron carrier on spheroids as a model of cancer cells is intended.

5. Summary

In conclusion, increase in cancer incidence and resistance to some treatments demands a new modality of tumor treatment or refinement of existing ones. One of the possible new therapies there is the BNCT, which needs an improvement in the boron delivery to the tumor cells. This can be reached through increasing of the selectivity of boron carrier to cancer cells by using a carrier with a nucleic acid complementary to the mutation in melanoma cells. Application of spheroids may provide a model as close as possible to the conditions prevailing in the tumor.

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