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Ultrasound induced cancer immunotherapy ☆

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ABSTRACT

Recently, the use of ultrasound (US) has been shown to have potential in cancer immunotherapy. High intensity focused US destruction of tumors may lead to immunity forming *in situ* in the body by immune cells being exposed to the tumor debris and immune stimulatory substances that are present in the tumor remains.
Another way of achieving anti-cancer immune responses is by using US in combination with microbubbles and nanobubbles to deliver genes and antigens into cells. US leads to bubble destruction and the forces released to direct delivery of the substances into the cytoplasm of the cells thus circumventing the natural barriers. In this way tumor antigens and antigen-encoding genes can be delivered to immune cells and immune response stimulating genes can be delivered to cancer cells thus enhancing immune responses. Combination of bubbles with cell-targeting ligands and US provides an even more sophisticated delivery system whereby the therapy is not only site specific but also cell specific.
In this review we describe how US has been used to achieve immunity and discuss the potential and possible obstacles in future development.

Contents

Abbreviations: AB, antibody; BL, bubble liposome; CTL, cytotoxic T lymphocyte; DC, dendritic cell; FUS, focused ultrasound; HIFU, high intensity focused ultrasound; hsp, heat shock protein; IFN, interferon; IL, interleukin; MB, microbubble; NK, natural killer cell; pDNA, plasmid DNA; PFC, perfluorocarbon; TNF-α, tumor necrosis factor α; T_{reg}, regulatory T cell; US, ultrasound.

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1. Introduction

Cancer is caused by the patient's own cells growing in an uncontrolled and harmful way. In general the immune response towards cancer is weak since the immune system sees the cells as "self". Furthermore, in cancer tissues the environment often suppresses the immune response by expression of receptors on the cancer cells and secretion of various immune suppressing substances [1].

In recent years there have been a number of reports that US can be used to boost immune response towards cancer. In this review we describe both the direct effect of US on tumors that can induce immune response and the use of US-sensitive drug carriers for delivery of immune-stimulating substances.

2. Overview of cancer immunotherapy

Immunotherapies are therapies where the natural immune response of the patient is activated or enhanced so that it acts to combat the disease. In cancer this means that the immune system should be made to attack the tumor or cancer cells but leave the normal, healthy cells alone. This can be done in different ways, by unspecific increase of the immune system, by using monoclonal antibodies, by adoptive cell transfer and by *in vivo* cancer vaccines [2]. Immunity can be divided into humoral immunity and cell-mediated immunity. Humoral immunity acts through antibodies (ABs) produced by B lymphocytes and cell-mediated immunity through T lymphocytes. Both types of lymphocytes can be activated by tumor antigens (TAs), which are mainly proteins and peptides from tumor cells. ABs are proteins that have affinity for a specific structure, for example a surface protein of a cancer cell. T cells are activated when the antigen is presented to them by major histocompatibility complex (MHC) molecules on cell surfaces. T helper cells (CD4+ cells) are activated by TA on MHC class II on antigen presenting cells (APCs), most importantly dendritic cells (DCs) and cytotoxic T cells (CTL, CD8+ cells) which can be activated by TA on MHC class I which is expressed on all cells [3]. CTLs can directly attack cancer cells showing the right antigens by releasing cytotoxins that lead to the death of the target cell. The T helper cells act by releasing cytokines, which is an important factor in CTL and B cell activation.

There are three steps essential for effective immune response against cancer [4]. Firstly DCs need to be exposed to TAs. The DCs also need to get a "maturation signal" that leads to immunity to the antigens instead of tolerance. Many maturation signals have been identified, such as pathogen associated molecular patterns, toll like receptor ligands, tumor necrosis factor α (TNF-α) and many more; however, the exact relationship between these signals is still not clear [5]. The second condition for immune response is T cell activation by DCs in the lymph nodes. If DCs that present antigens have not been activated by maturation signals they will instead induce tolerance in the T cells and thus counteract immune reaction [6]. The third step for effective immune action against a tumor is infiltration by the activated T cells in the tumor tissue and that they retain their activity and kill the cancer cells. The tumor microenvironment can also prevent the T cell effect in different ways, *e.g.* by the action of myeloid-derived suppressor cells and regulatory T cells (T_{reg}) that oppose the action of the activated immune cells and by the tumor cells down-regulating their MHC class I expression and release immune suppressing substances [1]. Immune therapies can act anywhere in this complex system but understanding the whole process will be essential for a successful outcome.

ABs are the immune modulating treatments against cancer most often clinically used today, and there are several approved products on the market [7]. Since ABs can be designed to bind to virtually any cell surface structure, they are very attractive tools for targeted treatment. In cancer therapy, ABs have been used to achieve targeted drug and radioisotope delivery and have been employed as immune-regulating agents. In immunotherapy, ABs can have several roles: (1) as a new antigen by binding to cancer cells and be discovered as non-self by immune cells; (2) as blocking agents of receptors involved in down-regulation of the activity of CTLs; or (3) oppositely be used to stimulate receptors that enhance the activity of immune cells [8].

Cancer vaccination can be performed in different ways. The simplest is the "classic" vaccine type where whole cancer cells removed by surgery or cancer cell line cells that carry some characteristic antigens of the cancer in question have been made non-viable by, for example, freeze-thawing or ultraviolet radiation. Then the cells or cell parts are injected into the patient [9]. This makes the TAs available for detection by DCs *in vivo* and can thus trigger an immune response. Another type of vaccination is adoptive cell transfer (ACT) in which activated anti-tumor lymphocytes are infused into the patient. The T cells are taken from the tumor tissue, tested for anti-cancer activity, expanded *ex vivo* to greater numbers and then re-infused into the patient [10,11]. DNA vaccination of tumor cells is another approach. Instead of directly potentiating the immune response towards the tumor, the tumor itself is made more immunogenic [12].

As mentioned there are several AB products on the market today. For example, Bevacizumab is an antibody that binds to vascular endothelial growth factor (VEGF) and prevents its function. VEGF is involved in the formation of new blood vessels in the tumor so blocking VEGF reduces this and thus the delivery of nutrients to the tumor [13]. Rituximab is another example, which is used in lymphoma where it binds to CD20 lymphoma cells and causes cell lysis and apoptosis [14].

When it comes to cancer vaccines there is only one substance approved today in the USA, Sipuleucel-T (or Provenge as it is known) induces targeting by the immune system of the antigen PAP and is approved for treatment of prostate cancer [15]. Sipuleucel-T is a cell-based therapy; cells are taken from the patient, cultured *ex vivo* with PA2024, a fusion protein where PAP has been conjugated with granulocyte-macrophage colony stimulating factor (GM-CSF). The cells are then infused back into the patient, discovered by the immune system and lead to immune response towards PAP which is expressed in about 95% of prostate cancers.

3. US

US is sound waves of frequencies from about 20 kHz and above, which is higher than can be detected by the human ear [16]. An US wave is created at the US transducer and propagates as intermittent high and low pressure zones through a medium.

3.1. Biological effect of US

Since US has a long history in medical applications the effects on biological tissues are well known. US used for *in vivo* imaging is generally considered safe but it is not completely without side effects (for a review see [17]). Adverse effects come primarily from two mechanisms: thermal effects and mechanical or cavitation effects [18]. Thermal effects are due to the absorption of the US energy. The amount of heating depends on both the US and the tissue exposed. From the US side, the energy of the US source, the tissue volume irradiated (*i.e.* concentration of radiation) and exposure time affect the heating. The heating of a tissue depends on the molecular composition, thermal conduction and blood perfusion.

Bubble destruction due to inertial cavitation can cause direct tissue damage through heat and jet streams from the collapsing bubble but