International Immunopharmacology xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Cancer immunotherapy *via* nucleic acid aptamers

Mostafa Khedri [a], Houshang Rafatpanah [a], Khalil Abnous [b], Pouria Ramezani [b], Mohammad Ramezani [b,*]

[a] Department of Immunology, Immunology Research Center, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
[b] Pharmaceutical Research Center, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran

ARTICLE INFO

Article history:
Received 2 August 2015
Received in revised form 9 October 2015
Accepted 13 October 2015
Available online xxxx

Keywords:
Aptamer
Cancer
Immunotherapy
Delivery system

ABSTRACT

Over the past decade, immune therapy has become a standard treatment for a variety of cancers. Monoclonal antibodies, immune adjuvants and vaccines against oncogenic viruses are now well-established cancer therapies. Immune modulation is a principal element of supportive care for many high-dose chemotherapy regimens. Aptamers are short nucleic acids that bind to defined targets with high affinity and specificity. The first aptamers have been selected around two decades ago by an *in vitro* process named SELEX (systematic evolution of ligands by exponential enrichment). Since then, numerous aptamers with specificities for a variety of targets from small molecules to proteins or even whole cells have been selected. Targeting immunomodulatory ligands in the progressive tumor lesions of the patients would be prophylactic or therapeutic and may reduce drug-associated toxicities. A new class of inhibitory and agonistic ligands composed of short oligonucleotide (ODN) aptamers was developed recently that exhibited bioactivities comparable or superior to that of antibodies. This paper addressed progress in cancer immunotherapy with nucleic acid aptamers and highlighted recent developments either in immune system targeting or in immunotherapy methods involved aptamers. We discussed aptamer limitations when used as therapeutic agents for cancer treatment and suggested ways to overcome those limitations.

Contents

1. Introduction . . . 0
2. Methods used for developing immunotherapeutic aptamers and technical challenges involved . . . 0
3. Immune costimulatory-based cancer therapy with aptamers . . . 0
3.1. CTLA-4 . . . 0
3.2. 4-1BB . . . 0
3.3. OX40 . . . 0
3.4. CD28 . . . 0
3.5. PD-1 . . . 0
4. Aptamers against immune-suppressive cytokines . . . 0
5. Bi-specific aptamers for cancer immunotherapy . . . 0
6. Aptamer-targeted antigen delivery in cancer immunotherapy . . . 0
7. *Ex vivo* and *in vivo* use of aptamers in cancer immunotherapy . . . 0
8. Conclusion . . . 0
Acknowledgments . . . 0
References . . . 0

1. Introduction

There are established therapies employing a variety of manipulations to activate antitumor immunity. One type constitutes passive immunization with monoclonal antibodies, the introduction of adjuvants into the tumor microenvironment, and the systemic injection of cytokines. However, active transfer of antigen-loaded dendritic cells, tumor-activated T cell, tumor antigens formulated with either adjuvant or included in different delivery systems comprise second approach. Currently, immune therapy has become a standard treatment for a variety of cancers, and in some instances, could be considered as a

* Corresponding author.
E-mail address: ramezanim@mums.ac.ir (M. Ramezani).

http://dx.doi.org/10.1016/j.intimp.2015.10.013
1567-5769/

replacement for nonresponsive cases to chemotherapy and other traditional strategies for cancer treatment [1].

Investigational immune therapies for cancer, involve devising more efficacious and less toxic cancer therapies upon established treatment regimens. A wide variety of novel strategies have been developed based on our understanding of the interactions between tumors and the immune system. Collectively, these strategies attempt to augment protective antitumor immunity and to disrupt the immune regulatory circuits that are critical for maintaining tumor tolerance [1]. The immune system has three basic roles in the prevention of tumors. First, it can protect the host from virus-induced tumors by eliminating or suppressing viral infections. Second is the timely and effective elimination of pathogens and prompt clearance of inflammation preventing the establishment of an inflammatory microenvironment conducive to tumorigenesis. Finally, some tumor cell express some antigens including tumor-associated antigens (TAS) and tumor specific antigens (TSA) which immune system can specifically identify them resulting in the elimination of tumor cells [2].

Aptamers are short nucleic acids (almost 12–80 nucleotides long) capable of specific and tight binding to their target molecules. The term aptamer is originated from the Latin word *aptus* (to fit) and the Greek word *meros* (part) [3]. Aptamer selection or isolation was accomplished by a process called SELEX (systematic evolution of ligands by exponential enrichment), which was first applied independently by Ellington and Szostak [4], Tuerk and Gold [5], and Robertson and Joyce [6] in 1990. A typical SELEX experiment starts with a library of up to 10^{15} random oligonucleotide sequences which can be DNA, RNA, or modified RNA (*e.g.*, 2-OMe or 2-F modified RNA). Some members of this rich library have strong affinity for binding to a desired target. Numerous aptamers (also termed Oligonucleotide Aptamer Ligands), have been developed during the past 20 years which can bind and inhibit the activity of many proteins. The concept that nucleic acid ligands could modulate the activity of proteins emerged from basic studies of viruses and early works in the field of gene therapy [7]. Research on HIV and adenoviruses in the 1980s, discovered that these viruses encode a number of small-structured RNAs that bind to viral or cellular proteins with high affinity and specificity. For example, the human immunodeficiency virus has evolved a short-structured RNA ligand (TAR). The HIV TAR element binds to the viral proteins such as tat, as well as the cellular protein cyclin T1. HIV uses the TAR element to control viral gene expression and replication. Adenovirus has evolved a short-structured RNA aptamer, termed VA-RNA, which inhibits interferon-induced PKR activity and thus blocks one of the antiviral strategies employed by mammalian cell's [7]. In the late 1980s, the observation that viruses utilize RNA ligands in their biological activities particularly for escaping from immune-responses suggested that RNA ligands might also be useful for therapeutic and diagnostic purposes. The first study in 1990 indicated that CD4 + T cells containing the TAR aptamer were highly resistant to viral replication and cytotoxicity. Thus, for the first time in these studies, it was demonstrated that nucleic acid aptamers could be used as an agent to directly bind and inhibit the activity of proteins, suggesting possible clinical outcome [8]. This approach has been widely used during the past two decades to generate RNA ligands for many proteins. In 2003, Gilboa et al. demonstrated that aptamers can be employed as a potential therapeutic agent for cancer treatment by targeting immune-receptors described below. His work offered a new strategy for cancer immunotherapy [9].

In this review, we describe aptamers which are implicated in the treatment of cancer, particularly those which target proteins involved in tumor immunotherapy thereby preventing tumor immune-escaping (Table 1). Aptamers are not only a new and promising alternative to antibodies in tumor diagnostics, but also can be used in direct tumor immunotherapy and delivery systems. Aptamer can be used directly for either therapeutic applications or delivering drug molecules to disease-related cells or tissues of interest thereby minimizing the exposure of these possibly harmful agents to surrounding healthy tissues (decrease bystander and adverse effect of the main drug). Aptamer immunotherapy can replace monoclonal antibody therapy. There are some promising examples of cancer antibody therapy which potentially can be replaced by aptamer therapy. Ipilumimab is a FDA approved antibody that blocks the inhibitory action of CTLA-4 [10,11], and clinical trials targeting 4-1BB and PD-1 or PD-L1, have demonstrated the therapeutic potential of using immunomodulatory antibodies to stimulate protective immunity in human patients [12]. Nonetheless, systemic administration of immunomodulatory antibodies has been associated with dose-limiting autoimmune pathologies, conceivably reflecting also the activation of resident auto-reactive T cells [8]. Aptamers have notable advantages over antibodies which include, a) as a cell-free chemical synthesis is used for aptamer selection, they are more simple and cost-effective in comparison to cell-based products like antibodies, b) conjugation of aptamers to other entities such as toxins or drug carriers is easier compared to antibodies, and c) antibody-based therapeutic agents have at least one foreign motif which may promote immune response in receiving hosts, whereas aptamers due to their nucleic acid nature and size, initiate less or no immune response. However, translating aptamers into clinic faces some major limitations such as fast renal clearance and digestion by serum nuclease [13] which are explained in the next section.

In traditional approach of cancer therapy by aptamers, they were mainly developed for targeting tumor markers. These include aptamers targeting MUC-1 (CD227) [14], prostate specific membrane antigen (PSMA) [15], human epidermal growth factor receptor-3 (HER3), tenascin-c [14,16] and some other receptors [17]. Among these, only aptamers specific for HER3 as one of the tumor markers, exhibited antagonistic activity [2]. Nowadays, aptamers are not only considered as a new and promising alternative to antibodies in tumor diagnostics

Table 1
Immune molecules targeted by aptamers for cancer therapy.

Target type	Target	Reactivity	Chemistry	Application	Reference
Costimulatory	CTLA-4	Murine	2′ F pyrimidine RNA	Antagonist	[9]
	CD28	Murine	N6-(6-aminohexyl) RNA	Agonist and antagonist	[53]
	OX40	Human	Dimer RNA	Agonist	[9]
	4-1BB	Murine/human	2′ F pyrimidine RNA	Agonist	[37]
	CD4	Rat and human	2′ F pyrimidine RNA		[100]
Adhesion molecule	VCAM-1	urine	2′ F pyrimidine RNA	Binding	[101]
	Selectin P	Human	2′ F pyrimidine RNA	Antagonist	[102]
	Selectin L	Human	ssDNA	Antagonist	[103]
	Sialyl Lewis X		RNA	Antagonist	[104]
Cytokines and chemokines	IFN-γ	Human	2′ NH2/F pyrimidine RNA	Antagonist	[105]
	TGF-β1	Human	Ss phosphorothioate DNA	Not defined	[106]
	TGF-β2	Human	2′ F/OMe RNA	Antagonist	[107]
	CXCL-10	Human/murine	2′ pyrimidine RNA	Antagonist	[108]
	MCP-1	Murine/human	2′ F pyrimidine RNA	Antagonist	[109]
	Oncostatin M (OSM)	Human	2′ F pyrimidine RNA	Antagonist	[110]

Please cite this article as: M. Khedri, et al., Cancer immunotherapy *via* nucleic acid aptamers, Int Immunopharmacol (2015), http://dx.doi.org/10.1016/j.intimp.2015.10.013