

## A new frontier: Unveiling the possibility of targeting intracellular antigens with nucleic acid aptamers

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### ABSTRACT

Proven RNA-based medications have elevated the demand for targeted nucleic acid-based agents. In this study, nucleic acid aptamers represented an appropriate class of molecules. They are single-stranded nucleic acid molecules that bind to a target molecule with high affinity and specificity. Aptamers have a number of advantages over antibodies including compact dimensions, rapid tissue penetration, heat resistance, denaturation resistance, broad spectrum of targets, ease of chemical synthesis, ease of chemical alteration, convenient storage, minimal immunogenic response, negligible variability across batches, and cost-effective production. Here, we evaluated whether we could use aptamers to target intracellular antigens and how they could be applied for delivering therapeutics or diagnostic agents to tumor or viral-infected cells.

**Keywords:** Intracellular antigens, nucleic acid aptamers, TCR-like antibodies

### INTRODUCTION

Nearly all the antibody-based drugs currently approved for clinical applications

primarily target extracellular antigens, specifically antigens on the cell surface or secreted by cells [1]. However, more than half of clinically significant targets are

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present intracellularly [1]. These intracellular targets include proteins with mutations such as transcription factors and signal transducers, which are unique to tumors and not expressed in normal cells (neoantigens) [2,3]. Due to their selective expression in tumor cells, neoantigens present a promising focus for immunotherapy approaches. Other intracellular targets include viral proteins in infected cells.

Various approaches exist for targeting intracellular antigens: (1) the use of antibodies targeting externalized antigens like Phosphatase of Regenerating Liver 3 (PRL-3); (2) deploying intracellular antibodies (intrabodies) that are either generated internally via gene therapy or produced externally and then delivered into the cell using nanoparticles or by fusion with a cell penetrating peptide to access the intracellular target; (3) utilizing T-cell Receptor mimic (TCRm) antibodies, also referred to as TCR-like antibodies [4]. TCRm antibodies are monoclonal antibodies specifically chosen via phage display or hybridoma technology to bind short antigenic peptides (consisting of 8-10 amino acids) originating from intracellular proteins. These proteins could be mutant proteins within tumor cells or viral proteins within infected cells. TCRm antibodies

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bind to these antigenic peptides presented on the cell surface in the context of Major Histocompatibility Complex (MHC) class I [4]. Notably, there have been reports of TCRm antibodies targeting antigenic peptides presented in the context of MHC class II on certain cells [5,6]. The TCR found on T cells also bind to this peptide/MHC complex, and this plays a vital role in the regular monitoring of the immune system [7-9]. In a manner similar to TCRs, TCRm antibodies exhibit specificity towards an antigenic peptide and are restricted to a specific type of MHC.

Aptamers are single-stranded DNA or RNA molecules that exhibit high affinity and specificity in binding to a wide range of targets, ranging from small molecules to entire cells [10]. These aptamers are derived from a large random library through the Systematic Evolution of Ligands by Exponential (SELEX) enrichment technology [10-14]. Aptamers offer numerous advantages over antibodies, including compact size, rapid tissue penetration, resistance to heat and denaturation, ability to target diverse molecules, ease of chemical synthesis and modification, convenient storage, minimal immunogenic response, consistency across

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batches, and cost-effective production [15-17].

Despite their nucleic acid nature, aptamers possess the capability to function as chemical antibodies in cancer immunotherapy. Several aptamers have been developed that specifically target secretory molecules (chemokines and cytokines), cell surface molecules such as co-stimulatory, adhesion, and tumor immunosuppressive induction molecules, and inhibitory and activation receptors on immune cells. These aptamers hold potential for use in cancer immunotherapy and may offer an alternative to traditional monoclonal antibody therapy due to their ability to overcome autoimmune responses [18,19].

Nearly all the nucleic acid aptamers currently in clinical trials for therapeutic applications exhibit specificity towards cell surface or secreted proteins. Likewise, nearly all the aptamers employed thus far for delivering therapeutic agents to target cells are designed to bind cell surface antigens [15-22]. However, there are some reports of recombinant aptamers, known as intracellular aptamers or intramers, which are expressed inside cells and selectively intracellular targets. These aptamers have been utilized as a tool for disrupting,

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regulating, or elucidating the function of intracellular target molecule [23-25]. Nonetheless, they are not suitable for targeted delivery of therapeutic agents.

Some aptamers have the capability to bind specific cell surface targets and internalize into the cell. These aptamers are selected through a specific cell-SELEX procedure, which involves a lysis step after incubating the random library with cells just before the amplification step to amplify internalized oligonucleotides. Alternatively, an *in vivo* SELEX procedure can be employed [26]. While these aptamers can be utilized for targeted delivery of therapeutic agents, it should be noted that they are specific to cell surface antigens rather than intracellular antigens, and the number of aptamers identified through these procedures is limited.

*Hypothesis*

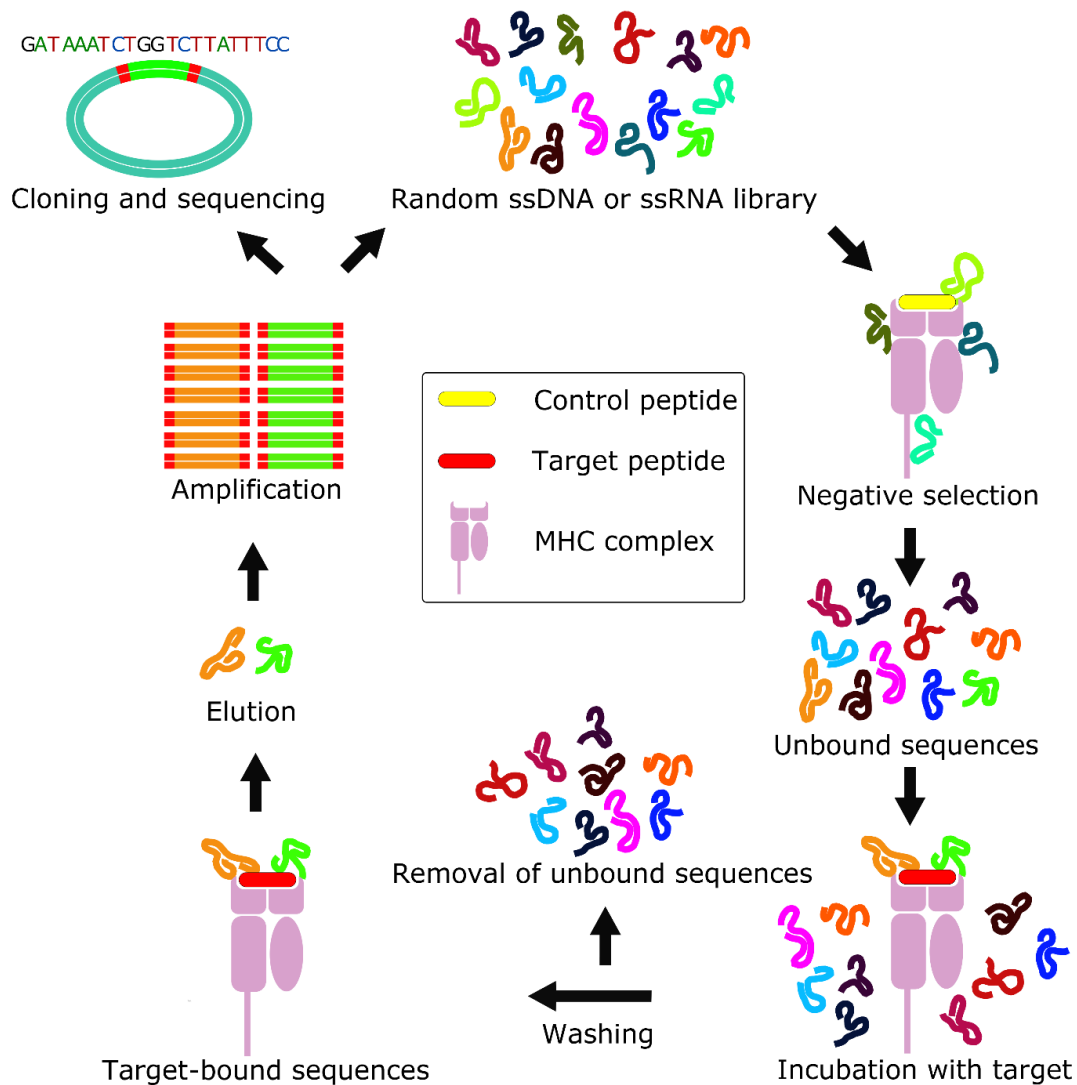
Here, we propose a hypothetical technique to identify specific aptamers for targeting intracellular antigens in cancer cells or viral-infected cells. This can be accomplished through a SELEX procedure where the target is an antigenic peptide-MHC complex, while non-specific peptide-MHC complexes serve as negative controls (Figure 1). In summary, a DNA or RNA oligonucleotide library is synthesized

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containing nucleic acid sequences that have a central random region and two short constant regions on either side. The library is exposed to one or more control peptide-MHC complexes to remove oligonucleotides that bind to the backbone MHC complex and control peptides presented in the context of MHC complex. Control peptides can be selected from naturally occurring proteins in the cell. The remaining unbound oligonucleotides are then exposed to the target peptide-MHC complex. The eluted oligonucleotides are amplified using PCR or reverse transcription PCR, depending on whether DNA or RNA molecules are being used.

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The resulting amplified DNA oligonucleotides are then converted to single-stranded form to generate the next round of the DNA library. If working with RNA libraries, a T7 promoter is usually present in one of the constant regions and is utilized for *in vitro* transcription by T7 RNA polymerase. Following multiple rounds of selection, the final enriched oligonucleotides are cloned and sequenced in order to identify the aptamers that have been specifically selected.



**Figure 1.** Schematic diagram of the proposed SELEX procedure for identifying aptamers specific to a target peptide-MHC complex.

The aptamers identified through this approach would be capable of recognizing the antigenic peptide derived from an intracellular protein when presented in the context of MHC at the cell surface. For simplicity, we can refer to these aptamers as TCRm or TCR-like aptamers, given

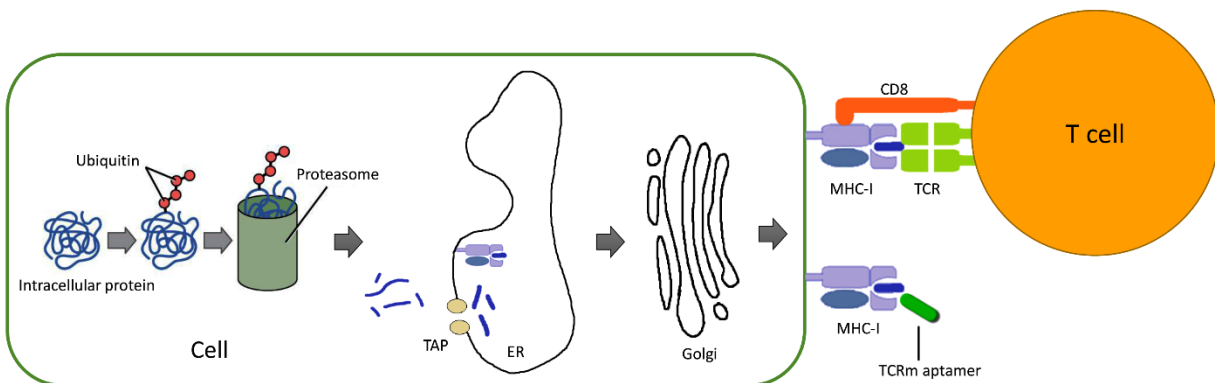
their resemblance to TCRs, which target intracellular antigens displayed on cells through MHC molecules (Figure 2). Table 1 shows selected advantages and disadvantages of TCRm nucleic acid aptamers and conventional nucleic acid aptamers that target cell surface antigens.

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The chances of successfully obtaining these aptamers appear to be higher compared to TCRm antibodies. Unlike antibodies, aptamers have a broad range of target molecules, spanning from small to large molecules. Remarkably, aptamers

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even possess the ability to distinguish a mutant protein with a single amino acid change from the wild-type protein [27].



**Figure 2.** The operational mechanism of T-Cell Receptor (TCR) mimic (TCRm) aptamers to target intracellular antigens. TCRm aptamers have the ability to recognize and bind to small peptides that are displayed by MHC molecules on the outer surface of cells. The TCR found on T cells also bind to this peptide/MHC complex, and this plays a vital role in the regular monitoring of the immune system.

**Table 1.** Selected differences between putative TCRm nucleic acid aptamers and traditional nucleic acid aptamers

Agent	Advantages	Disadvantages
TCRm aptamers	<ul style="list-style-type: none"> <li>▪ Access to intracellular antigens</li> </ul>	<ul style="list-style-type: none"> <li>▪ Possible cross-reactivity</li> <li>▪ Short plasma half-life</li> </ul>
Traditional aptamers	<ul style="list-style-type: none"> <li>▪ Well-characterized agents</li> </ul>	<ul style="list-style-type: none"> <li>▪ Limited to extracellular targets</li> <li>▪ Short plasma half-life</li> </ul>

## CONCLUSION

A theoretical SELEX procedure was proposed to identify specific aptamers that mimic T-Cell Receptors (TCRs) recognizing specific peptides derived from intracellular proteins presented in the context of Major Histocompatibility Complex (MHC) on target cells. These aptamers can be referred to as TCR mimic (TCRm) or TCR-like aptamers. They are analogues of TCRm antibodies and they hold potential for targeted delivery of therapeutic or diagnostic agents to tumor cells expressing an intracellular tumor-specific antigen. Furthermore, they can be utilized for directing therapeutic agents to

viral-infected cells expressing viral antigens. TCRm aptamers offer new possibilities in the field of immunotherapy by providing access to intracellular antigens for targeted delivery of therapeutic or diagnostic agents, while capitalizing on the advantages of nucleic acid aptamers over antibodies.

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