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RNAi nanomaterials targeting immune cells as an anti-tumor therapy: the missing link in cancer treatment?

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siRNA delivery targeting tumor cells and cancer-associated immune cells has been gaining momentum in the last few years. A combinatorial approach for silencing crucial factors essential for tumor progression in cancer-associated immune cells and in cancer cells simultaneously can effectively shift the tumor microenvironment from pro-oncogenic to anti-tumoral. Gene-therapy using RNAi nanomaterials can help shift this balance; however, fully utilizing the potential of RNAi relies on effective and specific delivery. RNAi nanomaterials can act as a Trojan horse which delivers siRNAs against immunosuppressive factors and reverses the regulatory activity of tumor immune cells residing in the tumor microenvironment. Here we review potential RNAi targets, means to activate and control the immune response, as well as ways to design delivery nanovehicles for successful RNAi immunotherapy.

Introduction

The archetype for cancer treatment is slowly changing from relatively nonspecific cytotoxic agents to selective mechanism-based therapeutics. The combination of immune-targeted gene silencing and other cancer therapeutics represents an untapped opportunity in cancer therapy and requires a deeper understanding of specific tumor mechanisms.

Tumor cells induce the infiltration of other cell types and instruct them (fibroblasts, endothelial cells and immune cells) in a cell-contact dependent (paracrine, receptor-mediated) as well as contact independent manner (endocrine, cytokines and other signaling molecules) to establish a self-promoting and mutually self-reinforcing tumor microenvironment (TME) that promotes tumor progression [1]. Tumor associated immune cells are major contributors to the TME as well as tumor growth and development, and their levels can be correlated to patient prognosis [2]. Modulation of this microenvironment represents the key for controlling tumor growth, as well as the development of metastasis. The enormous potential of targeting the immune system for improved cancer therapies was recognized as the 'Science breakthrough of the Year 2013' [3].

Several approaches to target tumor immunity are being explored, including (1) cancer vaccines [4,5], (2) immune cell checkpoint inhibitors and (3) specific immune cell depletion [6]. Cancer immunotherapy can be employed as a single therapy or in combination with therapeutics directly targeting tumor cells [7–9]. Targeting the immune system for anti-tumor responses has several advantages over therapies targeting tumor cells alone and especially over broad chemotherapeutic agents. In contrast to chemotherapeutics with dose-limiting toxicities and potential drug resistance in patients, re-programming cancer-associated immune cells to combat tumorigenesis is highly specific and able to induce a long lasting memory response [10].

RNAi technology, such as short interfering RNA (siRNA), has already been shown to modulate specific gene expression in cancer cells with subsequent tumor regression. Therefore, we believe that this technology should be extended to target immune cells, individually or as a combination therapy [11]. Despite their high potential, using naked siRNA molecules presents several

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challenges, such as extremely short half-lives (i.e. minutes), weak protection against nucleases poor chemical stability, and dissociation from the vectors used [12]. Therefore it is imperative to pursue appropriate design and construction of nanoparticles for safe and efficient siRNA delivery.

Nanotechnology offers versatile, targeted delivery platforms for RNAi therapeutics [13–16]. In the last decade the use of inorganic (quantum dots, gold, silica and magnetic nanoparticles) and organic nanoparticles (liposomes, lipids, dendrimers, micelles) as siRNAs delivery agents has been extensively described [17–23]. Therefore, using RNAi in conjunction with nanomaterials is a valuable tool to target immune cells for cancer treatments (Fig. 1). Despite its therapeutic potential, the application of RNAi to orchestrate immune responses has so far been overlooked, but likely represents a highly valuable tool to combat tumors and shift the tumor microenvironment from pro-oncogenic to anti-tumoral.

This review article focuses on cancer immunomodulation and therefore only includes studies in which RNAi nanomaterials were used to target cancer associated immune cells or both the tumor and immune cells simultaneously.

RNAi delivery: promises and challenges

RNAi (e.g. siRNA) is a ubiquitous, highly specific, endogenous and evolutionarily conserved mechanism used to modify gene expression and is increasingly being used for therapeutic applications. siRNAs are 21–23 nucleotide (nt), double stranded molecules (dsRNA), with symmetric 2–3 nt 3′ overhangs and 5′-phosphate and 3′-hydroxyl groups, that mediate the cleavage and subsequent degradation of complementary mRNA sequences and thus regulate gene expression [24,25]. The RNA silencing pathway begins when long dsRNA precursors are processed to siRNA duplexes by the RNase-III-like enzyme Dicer. These short dsRNAs are subsequently unwound and assembled into an effector complex, called the RNA Induced Silencing Complex (RISC) which can direct RNA cleavage, mediate translational repression or induce chromatin modifications [26,27]. The theoretical approach seems relatively simple; however, several hurdles have to be overcome to successfully introduce functional siRNAs into the target cell. Initially, the siRNA has to be transported to the desired tissue then penetrate a specific cell and finally be released into the cytoplasm.

The use of nanoparticles as carriers for siRNA has expanded dramatically during the last decade, revealing these materials as excellent candidates for gene therapy. Nanoparticles have the potential to replace viral vectors and their inherent disadvantages, such as toxicity and lack of specificity [28]. Extensive reviews about the toxicity of viral vectors can be found elsewhere [29–31].

Nanoparticles (NPs) have the ability to carry the siRNA to the target tissue and induce transfection. The intracellular delivery of nanoparticles remains challenging, however, extensive reviews of the progress made in this field can be found elsewhere [32–34]. Following successful internalization into immune cells, the siRNA can selectively modify immune responses dependent on the therapeutic target, the siRNA payload, or the route of administration [35]. Nanomaterials can be applied either systemically or locally; the route of administration plays a pivotal role in dictating the efficiency of these materials. In order to improve the efficacy of immunotherapies based on RNAi nanomaterials the route of administration must be carefully chosen and optimized (Fig. 2).

Systemic administration

Most therapeutic NPs are administered intravenously, which is generally associated with a strong immune response [11]. Intravenously injected nanomaterials are exposed to an extremely complex microenvironment of blood components immediately upon injection, which determines their biodistribution and ultimately their therapeutic efficacy. This route could potentially be used to

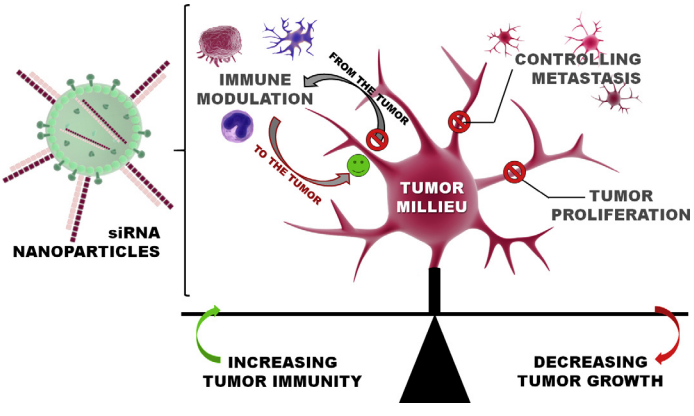

FIGURE 1

Tumors create a heterogeneous environment to promote their progression and suppress tumor immunity. Tumors are associated with infiltrating innate and adaptive immune cells. These cells aid tumor growth by creating an environment that disables immunogenic responses and assists in angiogenesis. Modulation of this microenvironment constitutes the key for controlling tumor immunity and tumor growth, as well as the development of metastasis. The delivery of RNAi nanomaterials is a promising new therapeutic to regulate immune responses and restore tumorigenic mechanisms.

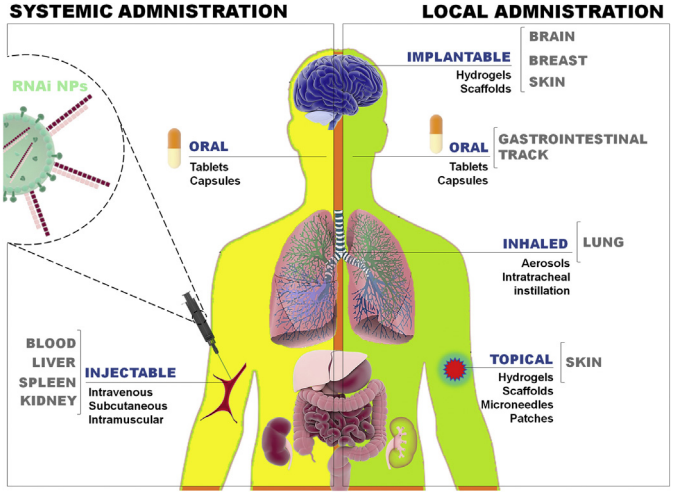

FIGURE 2

Routes of administration and potential target organs for RNAi nanomaterials. RNAi nanoparticles have to be applied in an organ dependent manner to reach the target tissue and consequently interact with the local immune or tumor cells.