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New Drugs

Current development of targeted oligonucleotide-based cancer therapies: Perspective on HER2-positive breast cancer treatment

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ABSTRACT

This Review discusses the various types of non-coding oligonucleotides, which have garnered extensive interest as new alternatives for targeted cancer therapies over small molecule inhibitors and monoclonal antibodies. These oligonucleotides can target any hallmark of cancer, no longer limited to so-called "druggable" targets. Thus, any identified gene that plays a key role in cancer progression or drug resistance can be exploited with oligonucleotides. Among them, small-interfering RNAs (siRNAs) are frequently utilized for gene silencing due to the robust and well established mechanism of RNA interference. Despite promising advantages, clinical translation of siRNAs is hindered by the lack of effective delivery platforms. This Review provides general criteria and consideration of nanoparticle development for systemic siRNA delivery. Different classes of nanoparticle candidates for siRNA delivery are discussed, and the progress in clinical trials for systemic cancer treatment is reviewed. Lastly, this Review presents HER2 (human epidermal growth factor receptor type 2)-positive breast cancer as one example that could benefit significantly from siRNA technology. How siRNA-based therapeutics can overcome cancer resistance to such therapies is discussed.

1. Introduction

The recent launching of the visionary Precision Medicine Initiative by President Barack Obama seeks to integrate individual variability in genes, environment, and lifestyle for personalized disease treatment and prevention. As the second most common cause of death in the US after heart disease, cancer remains one of the most fatal diseases. Oncology drug discovery is therefore at the forefront of the initiative. The initiative recognizes the issue of drug resistance and seeks to develop solutions. Progress in this regard will largely rely on programs such as the Cancer Genome Atlas (TCGA) project. TCGA researchers have begun to identify genomic aberrations and affected regulatory networks that enable aspects of cancer progression including proliferation, angiogenesis, invasion, drug resistance, and metastasis [1,2]. Unfortunately, many of the identified attractive therapeutic targets are considered 'undruggable' by conventional means (e.g., small molecule inhibitors, antibodies).

Advances in developing non-coding RNA molecules have provided a potential alternative strategy. RNA interference (RNAi) can easily be designed to modulate virtually any gene with a known mRNA sequence [3]. RNAi could also negate the dedicated costs and efforts associated with traditional chemical compound screening strategies in drug discovery. Knocking down expression of oncoproteins at the mRNA level is potentially a more effective approach because this process inhibits the synthesis of the active proteins, whereas monoclonal antibodies and small molecule inhibitors merely block their activity and do not halt the synthesis of new active oncoproteins. Despite their promise, few oligonucleotide-based therapies have reached the clinic due to inherent issues with bioavailability. The development of a versatile nanoparticle-based delivery platform is ever pressing for translating RNAi into the clinic.

This Review provides an overview on the various oligonucleotide technologies and nanoparticle delivery platforms that have reached clinical trials. Insight into the shortcomings and limitations of the first-generation delivery platforms and rationale for current state-of-the-art nanoparticle development will be discussed. Lastly, specific examples utilizing oligonucleotide-based strategies for treating HER2-positive (HER2+) breast cancer will

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be provided along with perspectives regarding their potential translational impact.

2. Non-coding oligonucleotides as therapeutics in cancer

The functional roles of oligonucleotides (nucleic acids), beyond their use in encoding genes and proteins, were discovered in the 1990s. The identified non-coding oligonucleotides were shown to have a role in regulating gene expression and cell function in all organisms [4]. Fig. 1 shows the research trend for each class of oligonucleotides and reveals that siRNAs and miRNAs have garnered the most interest since 2005. Although these oligonucleotides have promises in many disease applications, this Review will be limited to applications in cancer.

Small interfering RNAs (siRNAs) are small (19–25 nt) double-stranded RNAs, which are incorporated into a protein complex called RNA-induced silencing complex (RISC) upon cellular internalization [5]. Each siRNA has two strands, a sense strand and an antisense strand. The sense strand will be degraded by an endonuclease of RISC, argonaute 2. The antisense strand will guide RISC towards complementary target mRNA and induce mRNA cleavage. The siRNA strand containing the thermodynamically less stable 5′-end is preferentially incorporated as the antisense strand of RISC. Hence, chemical modifications are sometimes performed on siRNA strands to favor the incorporation of the intended antisense strand with RISC by modulating their thermodynamic asymmetry [6]. siRNA machinery (RISC) can also be recycled upon degrading each mRNA. Unlike antisense oligos (see next section), siRNAs have only one mechanism of gene ablation: mRNA cleavage. Although siRNAs and miRNAs share the same RISC-mediated RNA cleavage, siRNAs are optimized and designed to target certain genes with high specificity, while miRNA can have multiple or unknown targets (see next section). Thus siRNAs are deemed more effective and controllable than antisense oligos and miRNAs, and their pre-clinical and clinical investigations will be reviewed in the next sections.

miRNAs (mature microRNAs) are involved in regulating post-transcriptional gene expression and thus serve as one of the mechanisms that regulate cellular events and homeostasis [7]. miRNA mimics (small, chemically modified double-stranded RNAs that mimic endogenous miRNAs) follow the sequence of existing miRNAs presiding in various cell functions. Due to the ability to target multiple genes, the role of miRNAs in non-targeted cells can be uncertain. Further, miRNA expression can be upregulated or downregulated in cancer to promote cancer's survival advantages. Since miRNAs can behave as oncogenes or tumor suppressors [8], one can strategize with miRNAs therapeutically by either suppressing oncogenic miRNAs or introducing tumor suppressor miRNAs (e.g., miRNA mimics). The first miRNA mimic that entered clinical trial

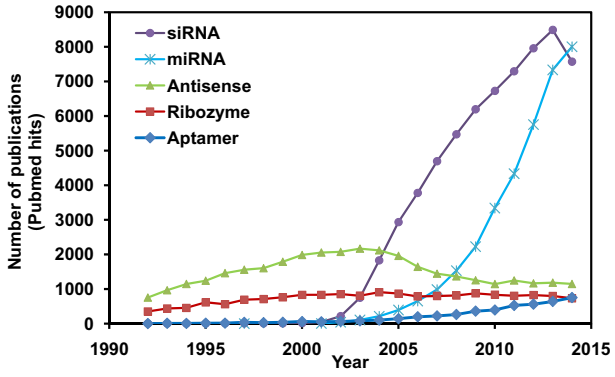

Fig. 1. Trend of research in oligonucleotides. The number of publications each year (1992–2014) based on Pubmed queries with specified keywords.

in 2013 utilizes a liposome-based technology to deliver miR-34a in cancer patients (primary or metastatic with liver involvement), exploiting their natural tendency to accumulate in the liver. The miR-34a was found to downregulate mRNA expression of several genes such as ERC1, RRAS, PHF19, WTAP, CTNNB1, SIPA1, DNAJB1, MYCN, and TRA2A [9]. This broad targeting ability can theoretically enhance therapeutic potential, but it also increases propensity for unwanted side effects.

Antisense oligonucleotides modulate gene expression by altering mRNA splicing pattern, blocking mRNA translation (by providing steric hindrance), and inducing degradation of targeted mRNA by the endogenous enzyme RNase H [4]. RNAs are inherently unstable in biological system due to the presence of nucleases and have poor pharmacokinetic profiles due to rapid kidney clearance. To overcome these issues, several chemical modifications of the antisense oligos have been performed. Phosphorothioate (PS) backbone modification is one of the earliest and widely used for oligos currently in clinics. PS modification increases the antisense's stability to nuclease degradation [10] and promotes binding to plasma proteins, which prevents rapid renal clearance and promotes uptake by certain cell types with scavenger receptors (e.g., kidney and liver cells) [11]. In newer generations of antisense therapeutics, additional modifications (e.g., sugar modification, base modification, direct conjugation to targeting ligands) are also performed in addition to PS to further improve their performance, as reviewed elsewhere [12]. One of the most advanced antisense oligos for cancer in clinical trials (i.e., reaching the NDA filing stage) is Genasense (Genta Inc.). Genasense was developed to block the production of the Bcl-2 protein, a key anti-apoptotic oncoprotein in cancer [13]. It was later rejected by the FDA for treating melanoma and chronic lymphocytic leukemia because the primary endpoint of improving overall survival was not met [14]. ISIS Pharmaceuticals is another leading company in antisense development. The most advanced antisense in their pipeline for cancer is OGX-011, which targets clusterin in castration-resistant prostate cancer. However, the phase III SYNERGY trial did not show significant improvement in overall survival [15]. Other next-generation antisense drugs for cancer developed by ISIS Pharmaceuticals include ISIS-STAT3-2.5Rx for targeting STAT3 [16] in hepatocellular carcinoma and lymphoma and ISIS-AR-2.5Rx for targeting AR [17] in prostate cancer. ISIS-STAT3-2.5Rx showed some clinical response in lymphoma patients (Phase I, 2014) and has currently progressed to Phase II studies [18]. ISIS-AR-2.5Rx is currently in the phase I/II stage, but there are no published results to date.

Ribozymes are considered self-processing RNAs in that they do not require proteins for catalysis. Angiozyme (Ribozyme Pharmaceuticals) is the first ribozyme that reached clinical trials for cancer treatment by targeting the vascular endothelial growth factor receptor-1 (VEGFR-1) in patients with renal cancer. Phase I results (2005) in patients with refractory solid tumors showed a favorable safety profile, and 25% of patients had stable diseases for more than 6 months [19]. Angiozyme was recently evaluated with metastatic breast cancer patients (Phase II, 2012) but did not show clinical efficacy [20].

Aptamers, unlike other non-coding RNAs, rely on their tertiary and quaternary structure for interacting and binding with target proteins [21]. Aptamers can bind proteins in a similar manner to antibodies but with less immunogenicity. Therefore, aptamers may serve as improved alternatives to current therapeutic antibodies. Like antibodies, most of the aptamer's targets are still confined to only extracellular or membrane proteins. AS1411 (Antisoma PLC) was designed to inhibit nucleolin activity and was the first aptamer to reach clinical trials for cancer treatment. Extended phase I (2006) and phase II (2014) studies have shown promising outcomes in patients with metastatic renal cell carcinoma [22].