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Review

Can gene editing and silencing technologies play a role in the treatment of head and neck cancer?

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ABSTRACT

Conventional treatment strategies have done little to improve the prognosis or disease-free survival in head and neck cancer (HNC) patients. Recent progress in our understanding of molecular aspects of head and neck squamous cell carcinoma (HNSCC) has provided insights into the potential use of molecular targeted therapies in combination with current treatment strategies. Here we review the current understanding of treatment modalities for both HPV-positive and HPV-negative HNSCCs with the potential to use gene editing and silencing technologies therapeutically. The development of sequence-specific RNA interference (RNAi) with its strong gene-specific silencing ability, high target specificity, greater potency and reduced side effects, has shown it to be a promising therapeutic candidate for treating cancers. CRISPR/Cas gene editing is the newest technology with the ability to delete, mutate or replace genes of interest and has great potential for treating HNSCCs. We also discuss the major challenge in using these approaches in HNSCC; that being the choice of target and the ability to deliver the payload. Finally, we highlight the potential combination of RNAi or CRIPSR/Cas with current treatment strategies and outline the possible path to the clinic.

Introduction

Head and neck cancer (HNC) is a major health problem and a leading cause of morbidity and mortality worldwide. It is regarded as the sixth most common cancer in the world. More than 90% of these malignancies are head and neck squamous cell carcinoma (HNSCC), which mostly comprise of oral squamous cell carcinoma (OSCC), oropharyngeal squamous cell carcinoma (OPSCC) and Laryngeal squamous cell carcinoma (LSCC) [1,2]. Tobacco use in different forms (including smoking and smokeless tobacco), areca nut and alcohol consumption are the most common risk factors for the development of HNSCC [3,4]. However, there is now irrefutable evidence of strong association of human papillomavirus (HPV) with HNSCC, especially for OPSCC in younger adult males [5,6]. Current treatment of HNSCC still involves conventional surgery, chemotherapy, and radiotherapy, or a combination of these. Radical surgical excision followed by radiotherapy, with or without adjunct chemotherapy, is now commonplace. Due to the complex and delicate anatomical structure of the face and neck, the extent of surgery may be limited, as wide excision results in visible and functional facial deformities: radiotherapy and chemotherapy have low specificity for individual neoplasms and have toxicities affecting the whole body [7]. HNSCC show a wide arrangement of genetic aberrations, increasing with tumour progression and these contribute to a therapeutic failure with conventional therapies [8]. Although in Western countries the average 5-year survival rate for HNSCC patients is now ~60%, one-third of patients develop local or/and regional metastases which reduce survival to less than a year [9,10]. It is clear that there is a need for the development of new treatment approaches, which would better preserve the patient's physical functions and their quality of life. Targeted molecular therapeutics are promising alternatives for treating cancer patients more efficiently and with limited side effects. One approach is blocking or silencing the expression of a specific gene (or genes) that is vital for continued growth of the neoplasm. Gene silencing or editing, via RNA interference (RNAi) and clustered, regularly interspaced, short palindromic repeats – associated with cellular apoptotic susceptibility protein (CRISPR/Cas) respectively, offer new possibilities for HNSCC treatment. The ability of

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RNAi to suppress the expression of target genes with high efficiency and specificity is well known. CRIPSR/Cas is a more recent discovery that allows the in situ manipulation of target genes but its clinical efficacy is yet to be proven [11]. The issue is which gene does one target and how will the therapy be delivered? Here we discuss gene editing and silencing mechanisms, potential candidate genes and review the various delivery technologies that could be used for HNSCC treatment. We highlight the potential path from bench to bedside for the improved treatment of HNSCC.

Gene editing and silencing

Both RNAi and CRISPR/Cas are programmable systems that allow one to specifically target a mRNA or gene, respectively. By providing each with sequence-specific RNA templates to their target, one is able to precisely change the output of that gene. RNAi achieves this via small non-coding RNAs such as short interfering RNA (siRNA), micro RNA (miRNA) or short hairpin RNAs (shRNAs) [12–14] and takes advantage of a normal cell regulatory system built into every eukaryotic cell. Structure-wise, siRNAs are 21 base pairs in length and are comprised of a 19 nucleotide duplex with a two nucleotide overhang at each 3′ end [15]. They can be introduced into cell cytoplasm in three different forms; (a) as synthetic siRNA or miRNA transfected directly into the cell [16]; (b) as longer dsRNA, transfected into cells, that are subsequently processed into siRNAs by the cytoplasmic enzyme Dicer (an RNase III endonuclease) [17], or (c) as plasmid DNA that expresses an shRNA, which is exported from the nucleus to cytoplasm and also processed by Dicer into an siRNA [18]. RNAi knocks down protein expression by functioning through three different cellular mechanisms. Firstly, it can inhibit transcription from genomic DNA by targeting complementary promoter regions through epigenetic silencing [19,20]. Secondly, it can inhibit the ribosomal translation of mRNA into protein [21]. The third and most well understood mechanism of RNAi is to form a cytoplasmic RNA-induced silencing complex (RISC) that causes destruction of the targeted mRNA [15,22], as seen in Fig. 1. In this mechanism, the single strand of RNAi (siRNA) has to be exactly complementary to the target mRNA.

The siRNA is loaded into the RNA-induced silencing complex (RISC) and the strands separated by the helicase activity with the antisense strand (complementary to the target mRNA) staying associated with the RISC. This then permits its binding with the homologous-targeted mRNAs through base-pair interactions, leading to endonucleolytic cleavage and subsequent degradation of targeted mRNA in the RISC by the action of the cellular exoribonucleases [23] (Fig. 1). Importantly, as the antisense-strand of siRNA is protected by the RISC from degradation, multiple mRNAs can be cleaved from a single siRNA [23]. This, therefore, allows RNAi to be used as a potential therapeutic tool for treating different diseases including viral infections, cancer and inherited genetic disorders.

Until recently it was though that adaptive immunity was a distinct feature only seen in eukaryotes. However, the recent discovery of CRISPR-Cas system suggest that the prokaryotes and archaea also possess a naturally occurring, complex and adaptive defence system [24]. CRISPR-Cas has gained much attention due to its powerful genome editing activity and possible therapeutic potential. The system is composed of a CRISPR – RNA sequence (crRNA), a

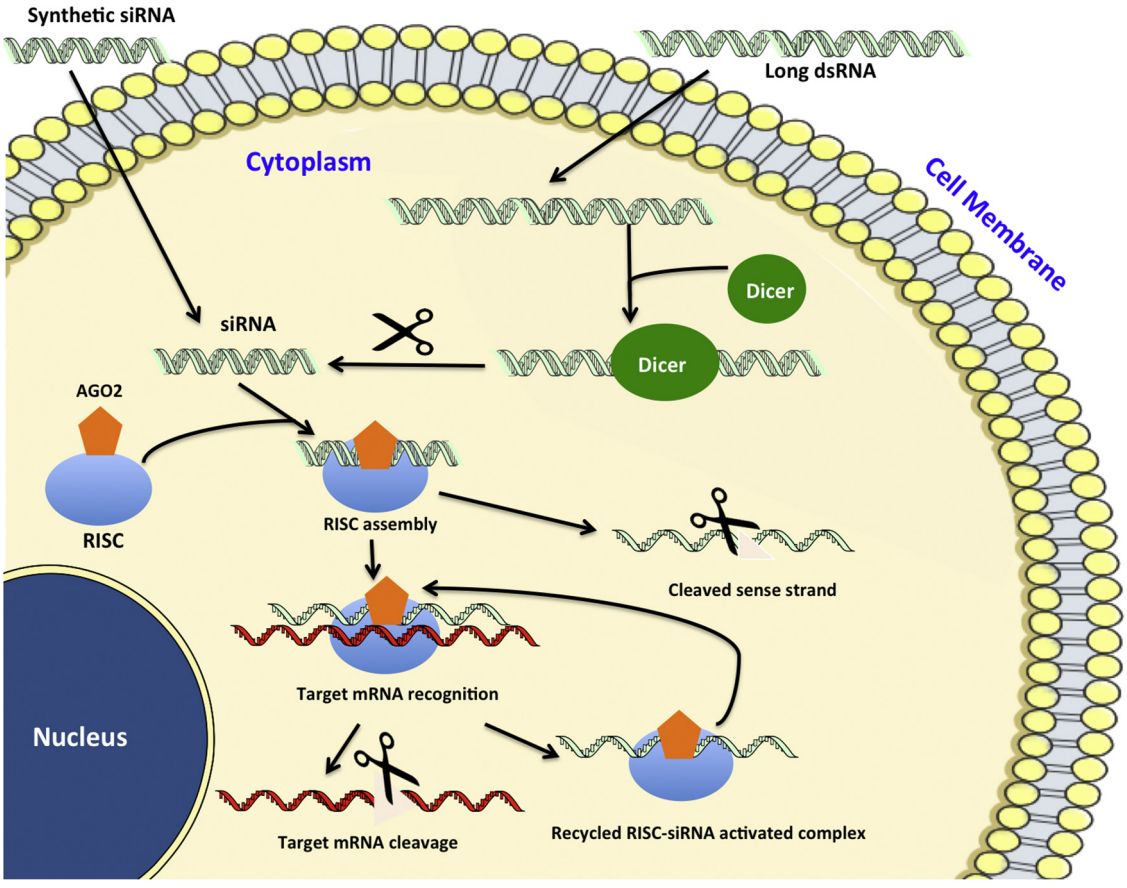

Fig. 1. The gene silencing mechanism of siRNA. The synthetic siRNA directly and long double-stranded RNA (dsRNA) processed by dicer to form siRNA into the cytoplasm. The activated siRNA associates with RNA-inducing protein complex (RISC) and mediates target sequence specificity for subsequent mRNA cleavage. This RISC complex is recycled and induces multiple rounds of silencing.