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Mini-review

Potential use of nucleic acid-based agents in the sensitization of nasopharyngeal carcinoma to radiotherapy

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ABSTRACT

Nasopharyngeal carcinoma (NPC) is a highly metastatic cancer. The 2-year survival rate of patients with stage III or IV disease is only about 50%. Due to its high radiosensitivity, radiotherapy is the standard treatment for early-stage of NPC. However, the radioresistance observed in some patients can cause distant metastases and local recurrence after radiotherapy. Special emphasis has been given to the discovery of effective radiosensitizers. Oncogenic proteins encoded by EBV genomes may serve as part of targeted radiosensitization, such as NF κB-mediated expression of latent membrane protein-1. We here review the major nucleic acid-based options currently used in cancer therapeutic approaches and the selected candidate genes that can be targeted for NPC radiosensitization.

1. Introduction

Radiation was first used to treat human diseases, including cancers, soon after Wilhelm Röntgen's discovery of X-rays in 1895. Radiotherapy is commonly used to control the growth of cancerous cells by employing high-energy beams of radiation to directly or indirectly damage targeted cells and tissues. Recent surveys have shown that 4 out of 10 cancer patients received radiotherapy at some time during the course of anti-cancer treatment. Nearly 500,000 patients in the United States receive radiotherapy each year as an essential part of their cancer treatment [1,2]. However, in spite of the many technological advances that have been made in the delivery of precise doses of radiation cancers, overall patient survival has not significantly improved. This is largely due to the side effects such as injury to normal cells and impacts on the long-term patient health and quality of life [3]. Cells that received smaller doses of radiation during earlier stages appeared to have reduced sensitivity to higher doses [4]. In this way, radiation therapy is often given alongside chemotherapy.

Nasopharyngeal carcinoma (NPC), a highly metastatic and invasive malignant cancer, originates from the epithelial lining of nasopharynx. NPC exhibits a marked geographic and racial distribution. There were an estimated 84,400 cases of NPC and 51,600 deaths in 2008, representing about 0.7% of the global cancer burden [5]. NPC is more common in male than in female patients in both the developing and developed world, with incidence rates commonly 2–3 times higher in male than female patients in higher-resource countries (Fig. 1) [5]. Distinguished by its unique clinical and pathologic characteristics, NPC is highly radiosensitive. Radiotherapy always serves as a primary treatment, achieving a 5-year overall survival of 90% and 84% for early stage I and IIA disease, respectively [6]. However, for advanced NPC, treatment outcomes have been unsatisfactory due to the significant rate of distant metastases and local recurrence after radiotherapy [7]. Combinations of ionizing radiation and conventional cytotoxic agents have shown some superiority over radiotherapy alone. Data from eleven randomized trials covering 2722 patients comparing radiotherapy with chemoradiation (1979–2001) showed an absolute benefit of 6% at 5 years in overall survival in favor of chemotherapy ($P < 0.0001$) [8]. However, the primary empirical applications of radio-chemotherapy typically lack detailed mechanistic explanations. Clinical use of most of these agents is often limited by unacceptable levels of normal tissue toxicity [9]. With the impressive improvements in the understanding of the molecular pathogenesis of NPCs, the molecules responsible for DNA-repair and radiation-induced signal transduction, and some genetic markers of normal tissue toxicity after radiotherapy are emerging as potent forces both in further characterizing important molecular pathways and in defining themselves as genuinely sustainable therapeutic targets. Effective

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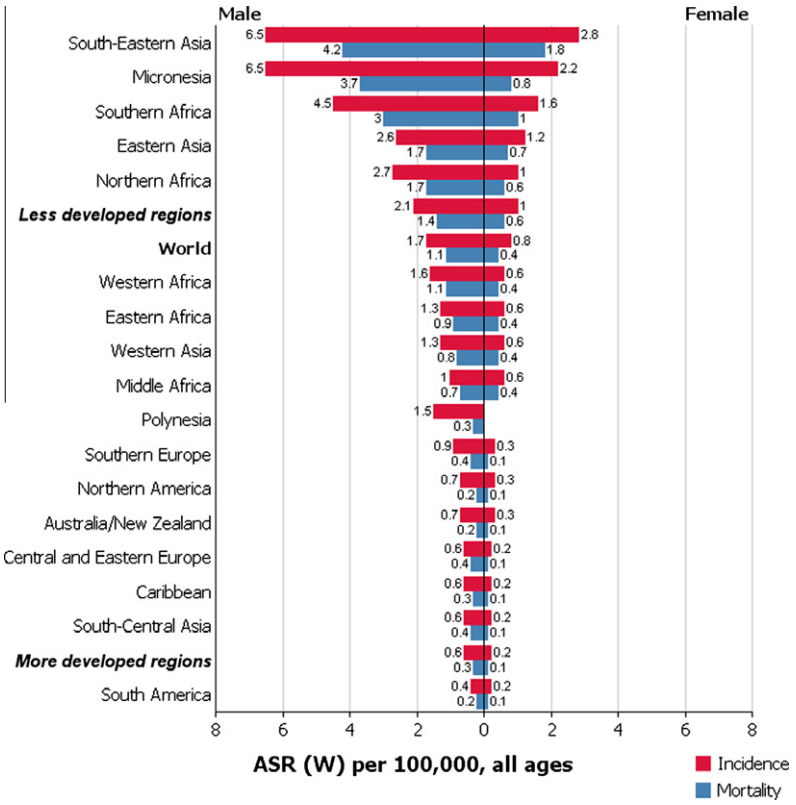

Fig. 1. Age-standardized nasopharyngeal cancer incidence rates by sex and world area. *Source:* GLOBOCAN 2008

molecular targets that can sensitize cancer cells to radiotherapy and protect surrounding normal tissues are urgently needed.

In this context, special emphasis is given to the exploitation of effective radiosensitizers that specifically target genes that may predict normal tissue toxicity after radiotherapy [10]. Chemical and pharmacological radiosensitizers include endogenous substances, such as oxygen, nitric oxide, and thiols, and xenobiotic chemicals that affect radiation damage in some way [11]. However, the use of chemotherapeutic agents and specific modifiers of radiation to enhance the radiation response is complex and frequently causes additional toxicity to patients. The heavy technical demands of effective drug delivery, the uncertainty of optimal timing of agents, and unique physiological micro-environmental conditions of individual patients must be thoroughly investigated before these agents may be used in practice [12].

Short nucleic acid-based cancer therapeutics encompasses a range of agents, including DNAzymes, short interfering RNAs, antisense oligonucleotides, and ribozymes. When engineered, these agents can modify the expression of the unwanted gene in a sequence-specific manner. They are considered powerful agents in silencing the resistance-associated genes in cancer therapy. However, the broad application of such cancer therapeutics is hindered by the efficient delivery of molecules to the target cells *in vivo*. In the following context, we review the major nucleic acid-based means with therapeutic potentials and the promising molecular targets for NPC radiotherapy. Combinations of such nucleic acid-based anti-cancer agents with radiation will also be discussed.

2. Nucleic acid-based gene-silencing agents

2.1. Antisense oligonucleotides

Antisense oligonucleotides (ASOs) are synthetic single-stranded segments of DNA or RNA generally 15–25 bp in length [13]. They have sequences complementary to their target mRNAs and inhibit the cognate gene product by specific sequence hybridization. They interact with the target mRNA, promoting physical blockage of the short double-stranded region or destruction of the DNA/RNA heteroduplex by cellular RNaseH. Both these mechanisms prevent translation [14]. Because unmodified ASOs are highly prone to degradation, ASO molecules are usually synthesized with phosphorothioate linkages, propyne analog, 2′-O-methyl RNA bases or latest locked nucleic acid (LNA) base analogs to enhance the stability and efficacy [15–17].

Previous reports have shown ASOs to specifically inhibit expression of many oncogenes and enhance the radiosensitivity of NPC cells. For example, ASOs against EBV latent membrane protein 1 (LMP1) and Epstein–Barr nuclear antigen 1 (EBNA-1) have been shown to inhibit viral oncoprotein expression, induce apoptosis, and sensitize the EBV-positive cells to cytotoxic agents [18–20]. Huang et al. used an ASO to specifically block the expression of BHRF1, an EBV lytic antigen with sequence homology to Bcl-2, in NPC cell line CNE2 [21]. They found that the treated CNE2 cells became more susceptible to radiation and showed weaker ability to proliferate, form colonies, and develop into tumors in nude mice after radiation. ASO was found to inhibit p21 protein expression and increase radiosensitivity in CNE-1-wtp53 NPC cells and prohibit tumor growth *in vivo* [22]. To date, several ASO drugs are in clinical development, most of which are phosphorothioate-modified (Table 1) [23]. These ASO drugs target the apoptotic rheostat, signaling pathways involved in cell proliferation and growth, or the cancer's microvasculature. Oblimersen (G3139), a Bcl-2 ASO, has a favorable safety profile in clinical trials for cancer therapy. Radiation was found to decrease the fraction of viable NPC cell line C666-1 to 60%, with a further decrease to 40% in combination with Oblimersen. This ASO combination with radiation also caused significant regression of established xenograft tumors in mice and extended survival time by 52 days in the case of mice with