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Review – Bladder Cancer

Synthetic Nucleic Acids as Potential Therapeutic Tools for Treatment of Bladder Carcinoma

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Abstract

Objectives: Abnormal gene activation in human tumours including bladder cancers (bCAs) may cause altered proliferation, maturation, and apoptosis as well as development of resistance to therapeutic interventions. Therefore, silencing of abnormally activated genes appears to be a rational approach for specific target-directed and sensitising therapies.
Methods: Of the available strategies for gene silencing, antisense-based techniques have attracted much attention and are the focus of this review. Putative target genes should be involved in essential tumour-promoting pathways, such as growth signalling, immortalisation, cell cycle regulation, apoptosis, angiogenesis, and development of therapy resistances. This review gives an overview of selected studies performed on bCA-derived cell lines and xenografts reporting down-regulation of potential target genes by antisense-based synthetic nucleic acids such as antisense oligodeoxynucleotides (AS-ODNs) and small interfering RNAs (siRNAs). Effects on proliferation of bCA cells and enhancement of the cytotoxic action of different chemotherapeutics were evaluated.
Results: Knock-down of the selected target genes frequently caused an impairment of growth of different bCA cell lines originating from cell cycle arrest or increased apoptosis. In numerous studies, the pretreatment with AS-ODNs or siRNAs provoked strong enhancement of subsequent chemotherapies, emphasising the effectiveness of these inhibition approaches.
Conclusions: The application of antisense-based inhibitors in combination with chemotherapeutics might represent an alternative strategy for the adjuvant treatment of superficial bCA. Nevertheless, translation of this technology to the clinic might be hampered by inestimable off-target effects caused by AS-ODNs and their behaviour after intravesical instillation has to be evaluated in preclinical and clinical trials.

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1. Therapeutic regimens for bladder cancer: standards and failures

Bladder cancer (bCA) is the second most common malignancy in urology with a high recurrence rate; 80% of these tumours are superficial at first diagnosis, but 10–70% of patients will have recurrence after transurethral resection and 10–20% of recurrent tumours are more invasive or less differentiated or both [1]. Intravesical treatment by early instillation of chemotherapeutics is being used effectively to reduce the recurrence rate [2]. Adjuvant intravesical therapy has been used for a long time, but only a few studies are available that conform to the stringent criteria allowing a scientific meta-analysis [3–5]. However, two facts seem to be clear: the rate of recurrence can be reduced by intravesical therapy and bacillus Calmette-Guérin (BCG) seems to be more effective in this than mitomycin C (MMC). It is equally clear that BCG also has more local and systemic side-effects than intravesical chemotherapy [5]. It also remains unclear which regimen of instillations is most effective and for how long a continued treatment should be given. At best, intravesical treatment can reduce the recurrence rate by 39% [6]. It therefore seems worthwhile to reconsider other forms of local, that is, intravesical treatment of bCA. This review gives an overview of a new option of such local treatment. The delivering of synthetic nucleic acids into superficially accessible malignant cells that interfere with tumour-specific molecular mechanisms offers a possibly useful and scientifically exciting new option of intravesical therapy. However, not all cells may take up the antisense oligodeoxynucleotides (AS-ODNs), possibly requiring their repeated application or a combination with other therapeutic agents.

The AS-ODNs and small interfering RNAs (siRNAs) can be constructed and delivered into remaining malignant urothelial cells after transurethral resection of a visible lesion. These constructs then interact with the complementary part of the DNA or RNA of the malignant cells and inhibit the expression of tumour-specific or related proteins, which results in enhanced cell death or reduced replication of the malignant cells. These strategies might be used on their own or in combination with intravesical instillation of conventional chemotherapeutics, enhancing their effects. These scientific approaches are at the stage of in vitro and animal studies (Table 1) and a few have been used in early human studies for other tumour entities. However, the unique possibility in bCA with the easy access to local application of agents working on malignant cells offers great potential for this form of treatment.

2. Nucleic acid inhibitors: mechanisms of action and therapeutic potential

The selective down-regulation of disease-related target genes by treatment with short synthetic nucleic acids is an attractive tool in molecular medicine including tumour therapy (for recent review, see Gleave and Monia [7]). Antisense-based synthetic nucleic acids are designed complementary to the mRNA sequence of the target gene and can hybridise with the mRNA by sequence-specific binding. Antisense strategies comprise (1) AS-ODNs, (2) siRNAs, and (3) catalytically active ribozymes and DNAzymes with an inherent RNA-cleaving activity [8]. The present review will focus on bCA-relevant experimental and clinical findings for AS-ODNs and siRNAs.

AS-ODNs consist of short single-stranded DNA normally 15–20 nucleotides in length. Three major mechanisms contribute to its activity (Fig. 1). Most AS-ODNs are designed to induce endogenous RNAse H activity, which cleaves the mRNA moiety of an mRNA–AS-ODN heteroduplex, and subsequently leads to its degradation. The binding of AS-ODNs may also cause the inhibition of translation due to steric blockade of the ribosome. Additionally, AS-ODNs can be delivered to the nucleus, where they can form a triplex with the double-stranded (ds) DNA sequence encoding the target mRNA and inhibit its transcription.

The siRNAs are synthetic dsRNA molecules typically 21–23 bp in length and known to down-regulate targeted genes by RNA interference (RNAi). Within the cytoplasm, siRNAs are incorporated into the RNA-induced silencing complex (RISC). The antisense strand of the siRNA together with RISC binds to the target mRNA and induces its degradation by the nuclease function of RISC (Fig. 1). Afterwards, the siRNAs can be reused for a further cleavage step.

One major challenge is the protection of AS-ODNs against nucleases by chemical stabilisation. Therefore, first-generation AS-ODNs contained phosphorothioate modifications. However, non-sequence-specific toxicities were mediated by the phosphorothioate backbone and the polyanionic nature of these compounds. Improved stability and enhanced affinity to RNA were achieved with second-generation AS-ODNs. Modifications include 2′-O-methyl or 2′-O-methoxyethyl (2′MOE) substituents at the ribose [7]. Locked nucleic acids (LNAs) provide increased hybridisation affinities [9]. Peptide nucleic acids (PNAs), which belong to the third-generation AS-ODNs, provide complete resistance to degradation [10]. The RNA-like conformation as