Antisense strategy in malignant brain tumours treatment

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Antisense approach

Malignant glioma, the most common human brain cancer, is almost uniformly fatal. Median survival is less than one year. The principal strategies of gene therapy for treatment of gliomas, including antisense approach, have been proposed comming from 1990s [1, 2, 3, 4].

The antisense oligonucleotides become the important tool of anti - cancer approach [5, 6]. The "discovery" of antisense approach was done by the groupes of R.M. Harland and of F. Jacob [7, 8]; the untranscribed DNA strand, that has been regarded only as a stabilizer and a protector of genetic material, was shown to reveal transcription activity [9]. It has also been widely proven that a lot of genes present an open reading frame on its antisense strand. Open reading frame on the antisense strand has been found in all genomes studied, both in prokaryotes and eukaryotes [10].

Different molecular pathways altered in cancer were exploited as potential the antisense strand has been found in all genomes studied, both in prokaryotes and eukaryotes [10]. On the basis of mechanism of action, two classes of antisense oligonucleotide can be discerned: (a) the RNase H-dependent oligonucleotides, which induce the degradation of mRNA; and (b) the steric-blocker oligonucleotides, which physically prevent or inhibit the progression of splicing or the translational machinery. The majority of the antisense drugs investigated in the clinic functions via an RNase H-dependent mechanism [5].

In prokaryotes and eukaryotes genetic information is supported by double-stranded DNA in which only one strand (sense strand) is usually transcribed to messenger RNA. The second strand is called the antisense strand because its sequence of nucleotides is the complement of message sense. This observation gave the origin to many antisense (as well as non-sense) approaches based on antisense RNA or antisense oligonucleotides, both targeting genes involved in pathological cellular processes. The antisense RNA is delivered to the cells either by a plasmid vector (dsDNA) encoding an antisense RNA or by a single sequence of nucleotides is the complement of message sense). The antisense RNA sequence is then produced by intracellular transcription of plasmid vector and is able to hybridize to the mRNA with subsequent translation blockade. Once hybridization occurs, the duplex RNA-RNA (DNA) stimulates ribonuclease H, the enzyme involved in DNA replication [11].

The first antisense oligonucleotide used in clinical pharmacology was as anti-cytomegalovirus therapy (VitraveneTM) [12]. The antisense strategy was then largely used in order to analyze gene expression and intron splicing. The most widely studied oligonucleotides are phosphorothioates, because their nuclease stability are highly soluble and have excellent antisense activity. These data have led to the introduction of phosphorothioate oligonucleotides into clinical therapeutic tumour trials [13, 14].

The triple helix (TH) technology is the new approach, which belongs together with antisense approach to anti-gene strategies. The TH technology was "discovered" by groups of P.B. Dervan [15] and of C. Helene [16]. So called triple-helix forming oligonucleotides, TFOs, are delivered to cells both by cell transfection with chemical carriers and via vector plasmid that can drive the synthesis of TFO RNA. TFOs link to genomic double-strand DNA, form triple-helix structure with target gene and strongly inhibit its expression at transcriptional level [15]. The role of 22-23 mer RNA in triple helix RNA-DNA mechanism is strongly similar to that of recent "siRNA technonology" involving also 23 mer RNA [16] (21-23-mer double-stranded RNA molecules, known as siRNA, can effectively silence gene expression [17]).

Oncogenes and genes encoding growth factors constitute the principal target of antisense strategy in malignant tumours treat-

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ment. The classical examples of use of antisense oncogens are that of c myb [18], bcr/abl [11], bcl-2 [19, 20, 21] or K-ras family [22], the last also explored in triple helix approach [23]. Growth factors and their receptors (that usually act as cell membrane tyrosine kinases) consist the complex system considered at present as especially important in oncogenesis. TGF-beta [24], EGFR [22, 25], VEGF [26, 27, 28, 29] represent relevant targets for anti-tumor gene therapies. Insulin-like growth factor (IGF-I) and its receptor IGF-I-R are considered as the most important growth factors related to the normal and neoplastic differentiation [30]. Therefor IGF-I antisense and IGF-I-R antisense gene therapies were proposed to treat different malignant tumours [3, 31].

Brain tumours and antisense approach

Neuroblastoma is the most common neuroectoderma derived solid tumour of paediatric age. C-myb gene expression has been reported in neuroblastoma. The use of antisense oligonucleotides as therapeutic antineoplastic agents has been recently investigated. It was demonstrated that the inhibition of cell proliferation was dependent on the down-modulation of c-myb protein expression [32].

Dysregulation of hMYCN protein expression appears to be critically involved in the pathogenesis of childhood neuroblastoma. Human neuroblastoma IMR-32 cells, which have an amplified hMYCN gene was transfected, with hMYCN AS [33]. The authors have examined the effects of continuous treatment for 6 weeks with AS oligonucleotides via subcutaneously implanted microosmotic pumps on tumor growth in a transgenic mouse model of hMYCN-induced neuroblastoma. Transgenic mice treated with AS oligonucleotides had lower tumor incidence and statistically significantly lower tumor mass.

Liposomes are one of the most promising delivery systems for genes, proteins, and other biological molecules and they are expected to become a new therapeutic tool for the treatment of brain tumors, especially malignant gliomas [34]. Recently, the promising results were showed using the strategy based on IGF-I antisense or triple-helix technologies and liposomes as delivery system for treatment of glioblastoma patients [35, 36]. The triple helix of IGF-I consists with single RNA strand containing a 23-nucleotide (nt) oligopurine sequence capable to form triple-helix structure with an IGF-I gene oligopurine/oligopyrimidine promoter segment. The injected triple helix IGF-I "vaccine" has developed T CD8 mediated immune response [37]. The interesting approach was also proposed by the group of R. Baserga, using antisense of IGF-I receptor 1 in clinical treatment of brain tumours (2003, personal communication); however the supposed anti-cancer immune response was not demonstrated in that approach [3].

The impact of bcl-2, a key antiapoptotic protein, on malignant gliomas by suppressing its expression was also investigated: antisense human bcl-2 cDNA was transfected into human malignant glioma cells. Transplantation of antisense bcl-2 cells resulted in no tumor formation [38]. Antisense bcl-2 expression could effectively reduce glioma survival, including retarding in vitro growth, complete loss of tumorigenicity, and significantly enhanced cisplatin cytotoxicity.

Human Nr-CAM (Neuroglia related Cell Adhesion Molecule) is over expressed in glioblastoma. Subcutaneous injection of antisense hNr-CAM overexpressing glioblastoma cells into nude mice caused complete inhibition of tumor formation [39]. Intra-tumoral inoculation of antisense hNr-CAM expressing plasmid also caused slow tumor growth in nude mice in vivo. The authors concluded that hNr-CAM is a valid target for potential gene therapy of glioblastoma tumors.

Some experimental models of glioma treatment were recently developed targeting different factors as telomerase, urokinase-type plasminogen activator receptor or matrix metalloproteinases. Telomerase is a ribonucleoprotein enzyme that is detected in the vast majority of malignant gliomas but not in normal brain tissues. Thus, antisense against human telomerase RNA component (2-5A-anti-hTER) was investigated for its antitumor effect on an intracranial malignant glioma model in nude mice [40]. The authors demonstrated that 2-5A-anti-hTER reduced the viability of malignant glioma cell lines to 20-43%. The treatment of intracranial malignant gliomas in nude mice with 2-5A-anti-hTER was therapeutically effective.

The urokinase-type plasminogen activator receptor (uPAR) and the p16 tumor suppressor gene play a significant role in glioma invasion. It was demonstrated that downregulation of uPAR and overexpression of p16 using a bicistronic caused an additive and cooperative effect in the suppression of the tumor growth of glioblastoma cell lines in an ex vivo intracerebral tumor model [41].

Increased expression of matrix metalloproteinases (MMPs) has been associated with human glioblastoma tumor progression. For this reason down-regulate MMP-9 expression was done by stably transfecting a high-grade glioblastoma cell line with a plasmid vector capable of expressing an antisense transcript complementary to a 528-bp segment at the 5' end of human MMP-9 cDNA [42]. Intracerebral injection of antisense stable transfectants in nude mice produced no tumors. These results suggest that MMP-9 expression is essential for the invasiveness of glioblastoma cells.

Conclusions

Human gene therapy is defined as a medical intervention based on the administration of genetic material in order to modify or manipulate the expression of a gene product or to alter the biological properties of living cells. Cells may be modified ex vivo for subsequent administration or altered in vivo by gene therapy products given directly to the subject. Example that falls under this definition include use of antisense oligonucleotides to block gene transcription or use of sequence-specific oligonucleotides to correct a genetic mutation [43].

The gene therapies in comparison to surgery or chemotherapy are new, sophisticated and still experimental. A number of strategies for inhibiting gene expression have been developed: the triple helix approach, decoy transcription factor binding and oligodeoxynucleotides seek to disrupt gene expression at the level of transcription. The antisense oligonucleotides and short interfering RNA molecules attempt to disrupt expression at the level of mRNA translation [44, 45]. Antisense therapy has been widely used to specifically and selectively inhibit the expression

of selected genes at the messenger RNA level. Combinations of antisense oligonucleotides with chemotherapeutic agents may offer important advantages in cancer treatment [46]. Anti - gene therapies are the subject of many clinical trials. It is necessary to underline their high specifity, relative security and very promising results. For that reason we hope, that this modern type of treatment [47, 48, 49] will soon become alternative for more traditional methods used in cancer therapy including therapeutic strategies for brain tumors [50].

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