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The diagnosis and therapy of brain tumours

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Abstract

Neoplasms arising from glial cells make up the most common group of primary brain tumors. The clinical outcome, especially the survival rates of the patients with brain tumours depend on tumour grade expressing its malignancy. A prognosis for glioblastomas (WHO IV) is very poor, but for astrocytomas (WHO I and II) it is relatively favourable. For oligodendrogliomas a longer survival time than for glioblastomas is observed.

There is evidence that oxidative stress and reactive oxygen species (ROS) are crucial in the etiology and progression of a number of human diseases, including neoplasms. An oxidative damage of DNA, lipids and proteins is caused mainly with hydroxyl radical ($^{\bullet}OH$), the most reactive ROS species and may be seriously deleterious.

In addition to all four basic nucleotides: adenosine (A), guanosine (G), tymidine (T) and cytosine (C), 5-methylcytosine (m^5C) is a rare but normal component of cellular DNA and occurs mainly within a sequence of a structural gene or in regulatory regions. In the reaction with hydroxyl radical all DNA components can be modified, but m^5C is relatively easily deaminated to thymine, which, in turn, pairs with adenine and after a round of replication, CG to TA transition occurs. Because thymine is a normal DNA base, therefore the product of spontaneous deamination of m^5C is not so easily detected by cell's DNA repair system. Thus, 5-methylcytosine residue constitutes a mutational hotspot and DNA methylation pattern in patients might be useful as a primary diagnostic tool or as a marker for early detection of relapse of the disease.

In recent years a new mechanism of posttranscriptional gene silencing has been discovered and named RNA interference (RNAi). This phenomenon is based on mRNA degradation mediated by small double-stranded RNA molecules, approximately 19-28 nucleotides in length, called short interfering RNA_s (siRNAs). These molecules are produced from long dsRNAs by a dsRNA-specific endonuclease (DICER) and form 300 kD multi-enzyme complex (RISC) which by Watson-Crick base-pairing of noncoding strand with their mRNA-targets induce specific degradation. The high sequence-specificity of RNAi makes it a new, promising tool in a gene-function analysis as well as in potential therapeutics development.

Key words: *brain tumours, epigenetics, 5-metylcytosine, RNA interference*

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Introduction

Understanding molecular mechanisms that occur in a normal cell and their possible ways of disregulation that lead to cancer is the prerequisite step in developing an anti-cancer therapy. After human genome sequence had been solved, it became obvious that not only alterations in the gene sequence can be deleterious, but also a damage of the epigenetic control of cell processes. There are several mechanisms of epigenetics: DNA methylation; histone methylation, acetylation and phosphorylation; RNA interference; chromosomal silencing via binding protein complexes or small RNAs; transposition of mobile elements. In this article we will discuss briefly the diagnostic potential and therapeutic application of DNA methylation and RNA interference in brain tumours.

DNA methylation

DNA methylation is a regulatory mechanism of gene activity. This process involves formation of 5-methylcytosine (m^5C), which function is a silencing or activation of a gene expression. It is an epigenetic marker and is inherited independently of the nucleotide sequence of DNA. Only 5% of all DNA cytosines are methylated, most of them (70-80%) in CpG islands. CpG dinucleotides usually occur at the 5'-ends of many human genes, most common in promoters and first exons, as well as at the 3'-ends. There are ca. 29 000 CpG islands in the human genome. They are combined with ca. 60% of human genes [1,2,5,14,18,25]. During cancerogenesis both global hypomethylation and local hypermethylation of CpG islands can occur, and that is the basis for the neoplastic process. These changes can be silencing of suppressor genes, a loss of gene imprinting, oncogenes activation, a higher number of point mutations in CpG islands and microsatellite DNA instability [15,22]. Partially a disturbance in the methylation pattern can be due to overexpression of DNA methyltransferases [22].

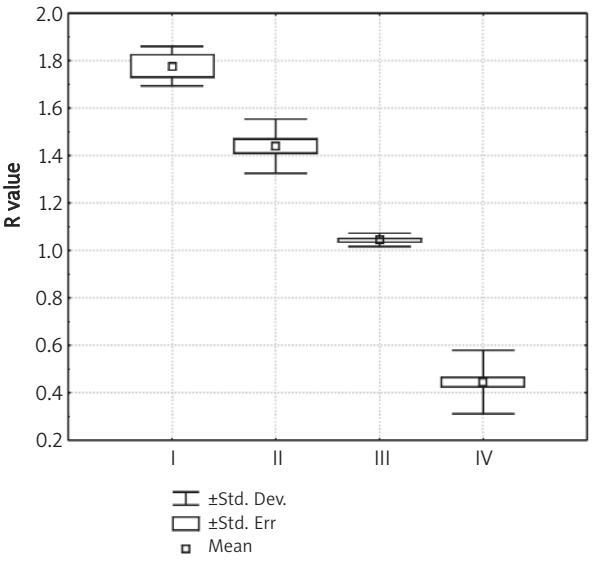

Fig. 1. The relation between R value for glial brain tumors and their malignancies (WHO I-IV). The statistical significance (ANOVA test) is F=369.33 and P=0.0000001. Tukey's Honestly Significantly Difference Test shows significant differences between all groups of tumor (P<0.0002-0.0003) [35]

Molecular markers

The genetic code is the first level of transmission of the hereditary information encoded in the nucleotide sequence. However, there are genetic variations that occur without corresponding changes in DNA sequence. These are called epigenetic and involve informational abilities of nucleic acids, proteins and chemical groups modifying them [5]. Genetic changes occur when nucleic bases are modified e.g. in the reaction with genotoxic chemicals and reactive oxygen species (ROS). However, a damage of m^5C with hydroxyl radical ($^{\bullet}OH$) beyond being a source of various modified nucleotides can also lead to thymine or cytosine, that are normal DNA bases. The final effect of this modification is global hypomethylation, which we analysed using chromatographic separation of [^{32}P]postlabelled components after enzymatic hydrolysis of tumour DNA [29]. We have found that there are significant differences in the content of m^5C in DNA of various tumour types: the lowest for WHO grade IV linearly rising while lowering the grade (Fig. 1). That finding makes the m^5C an epigenetic marker of DNA damage, which can be used for diagnosis and prognosis in brain tumours.

Silencing with RNA interference

RNA interference (RNAi) is an epigenetic regulatory pathway that serves as a sequence-specific

gene-silencer. Besides the role of the defence mechanism of eukaryotic cells against viruses and transposons, RNAi regulates the expression of homologous target-gene transcripts [12]. But sometimes the application of that technology in vertebrates, including mammals, is difficult because of additional dsRNA-triggered pathways that mediate a non-specific suppression of gene expression [7,19,21,27]. However, these non-specific responses are observed in the case of long dsRNAs but not short-interfering RNAs (siRNAs) [6,8,10,28]. siRNAs therefore seem to be promising reagents for developing gene-specific therapeutics [24]. siRNAs are 19-28nt dsRNA duplexes sometimes with symmetric 2-3nt 3' overhangs, and 5'-phosphate and 3'-hydroxyl groups [11]. This structure is characteristic of an RNase III-like enzymatic cleavage pattern, which led to the identification of the highly conserved Dicer family of RNase III enzymes as the mediators of dsRNA cleavage [3,4,17]. The process of degradation of the target messenger RNAs is restricted to cytoplasm [13,16,26]. In the first step, Dicer cleaves long dsRNAs to produce siRNAs, which are incorporated into a multiprotein RNA-inducing silencing complex (RISC). There is a strict requirement for the siRNAs to be 5' phosphorylated in order to enter the RISC [20,23]. siRNAs that lack a 5' phosphate are rapidly phosphorylated by an endogenous kinase [23]. The duplex siRNA is unwound, leaving the antisense strand to guide the RISC to its homologous target mRNA for endonucleolytic cleavage. The target mRNA is cleaved at a single site in the centre (10 nt from the 5' end of the siRNA) of the duplex region between the guide siRNA and the target mRNA [10,11]. siRNAs for gene-targeting experiments can only be introduced into cells via classic gene-transfer methods, such as liposome-mediated transfection, electroporation and microinjection, all of which require the chemical or enzymatic synthesis of siRNAs [9]. The efficiency of siRNA uptake is dependent upon the cell type. siRNAs can be synthesized in large quantities and, thus, can be used to analyse large numbers of sequences emerging from genome projects. siRNA-mediated RNAi is a powerful tool for regulating gene function.

We decided to use dsRNA for suppression of human glioblastoma multiforme by inhibition of the expression of tenascin C (TN-C)[21]. The protein is coded by a single gene and its expression is regulated by a single promoter. TN-C mRNA consists of 7560 nucleotides. The sequence length of 164 nucleotides was selected by computer calculations and match TN-C mRNA close to its 5' end. ATN-RNA was administered directly. In all cases postoperative wounds healing was *per primam intentionem*.

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