111

Design of effective antisense - oligonucleotides for science and medicine

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Antisense nucleic acids are widely used to suppress gene function in biomedical research and are developed as a molecular tool for clinical application.
Although the successfull inhibition of gene expression by antisense nucleic acids including antisense oligonucleotides (asON) has been demonstrated several times *in* cell culture and *in vivo*, a significant number of approaches in the use of this technology failed to show positive results and convincing therapeutic exploitations of asON still remain open. The selection of effective asON species out of the sum of all possible asON species against a given target still represents a major hurdle in the overall success in this field.
We have developed a new technology to design effective asON inhibitors which leads to a significantly increased rate of success.
This method is based on a combination of computer algorithms allowing high throughput, and the inclusion of empirical rules derived from experimental data. Both components enable a detailed analysis of the target RNA as well as of the antisense species.
The efficiency of this procedure to identify effective asON is reflected by an 10 times higher rate of succes in comparision to the use of random selected asON.

112

Tumor immune therapy targeted against onconeuronal antigens: Might this approach induce encephalomyelitis?

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Background and Goals: Antibodies directed against onconeuronal proteins such as anti-Hu are markers for a positive tumor prognosis. Furthermore, DNA vaccination with the onconeuronal protein Hu have been shown to reduce growth of an Hu-positive tumor in mouse model. These animals had a high anti-Hu titer in serum, but did not show an encephalomyelitis as side effect which would argue for using these onconeutonal antigens as targets for a specific tumor immune therapy. We now wanted to know whether a T cell transfer of T cells specific for the onconeuronal antigen Ma1 (Dalmau et al., Brain, 1999; Voltz et al., NEJM, 1999) could induce encephalomyelitis.
Methods: Rat Ma1 (rMa1) was cloned by RT-PCR initially using primers specific for the human Ma1 sequence and was completed by 5'and 3' genome walking. rMa1 was cloned into the expression vector pQE60, and recombinant protein purified by metal chelate chromatography. Female DA rats were immunized with recombinant rMa1 and rMa1-specific T cell lines generated from the draining lymph nodes ten days p.i. Freshly activated T cell blasts were transferred into naive female DA rats which were sacrificed 8 days later.
Results: Rat Ma1 is 93% homologous to the human Ma1 (corrected sequence) at amino acid level. Proliferation assays demonstrated that the CD4 positive T line cells were highly specific for rMa1 using a second recombinant antigen to control for reactivity with contaminating bacterial proteins. After T cell transfer the recipients did not show any clinical signs of disease, although they developed an intense perivascular inflammatory response in the brain stem and spinal cord. The distribution of lesions, in particular the involvement of the brain stem reproduces that seen in patients with paraneoplastic CNS disease associated with an anti-Ma1 antibody response.
Conclusion: T cells specific for an onconeuronal antigen may indeed induce an inflammatory response in the CNS. These data point towards the possibility of inducing an encephalomyelitis as side effect when using T cells specific for onconeuronal antigens for tumor therapy.