



Light-controlled DNA therapeutics may fight choroidal melanoma

Scientists at INSERM in Paris have recently demonstrated the potential of a novel gene delivery system for the treatment of choroidal melanoma.

The research findings utilise a well known herpes simplex viral protein known as "VP22" to deliver so-called "antisense oligonucleotides" to treat the cancer. Antisense oligonucleotides – or "ODNs" for short – are short stretches of nucleic acids that can bind to a complementary sequence and thereby interfere with the biology of a particular gene.

Once transported into cells, the ODNs can be activated by exposure to light. Although in its early stages of investigation, the findings are significant. In particular, the study's authors comment that "the light controlled delivery of ODNs to the intraocular structures is of particular interest due to the wide potential for the use of lasers in ophthalmology."

Details of the study appeared in the journal *Molecular Vision* at 2005;11: 184-191.

By way of background, VP22 is part of the Herpes Simplex Virus Type 1 protein. The Herpes Simplex Virus Type 1 is most commonly recognised in facial infections. The virus resides in a resting state in the nerves that run directly to the facial skin around the mouth of an infected person.

Stabilising for delivery

The C-terminal amino acids of the VP22 protein, when purified, are capable of binding ODNs to form small spherical particles ranging from about 0.3 to 1.0 micrometres in diameter. Once delivered into cells, the particles can remain stable for weeks at a time. The ODNs essentially act as highly specific drugs to inhibit genetic targets involved in the proliferation of cancerous cells.

A major challenge for the use of ODNs is to supply them in such a stabilised formulation that sufficient quantities of ODNs can survive the hostile cell environment and accomplish their therapeutic task. Fusing the ODNs to the VP22 protein provides such stabilisation, thus ensuring that sufficient amounts of the drug can reach the target.

In the case of the recently published studies, the target of the ODNs was a gene known as "c-raf kinase," which is involved in a cell pathway that transforms a normal cell into a cancerous cell. The research group explained the choice of this particular target because the gene "is unregulated in many human tumours and plays a pivotal role in cell proliferation and resistance to apoptosis." Thus, if ODNs could stop the c-raf kinase gene from expressing itself, then the researchers could inhibit the growth of the choroidal melanoma cells.

De-stabilising for therapeutic effect

In theory, once the mixture of VP22/ODNs could be delivered to its target area, researchers would then apply a cold halogen light or helium neon laser to the infected cells. The illumination would cause the mixture of VP22/ODNs to become destabilised. Once destabilised, the mixture would release the bound ODNs which could then bind to their anti-sense target. In this case, the target was the messenger RNA of the c-raf kinase gene that is involved in the proliferation of ocular choroidal melanoma.

Against such a background Drs. Francine Behar Cohen and Nadia Normand of the Laboratoire d'Innovation Therapeutique en Ophthalmologie A de Rothschild and INSERM then began to test their theory.

The researchers began by purifying the C-terminal half of the VP22 protein from bacterial cultures and mixed the final preparations with ODNs of 20 bases in length to form light sensitive complex particles known as "vectosomes."

The research group used two cell lines to test the vectosomes. One cell line was that of the human choroid melanoma cell line. The second cell line was that of the human retinal pigment epithelium cell line.

Cultures of the choroid melanoma cell line and retinal pigment epithelium cell line were incubated with vectosomes overnight where, in the absence of illumination, the vectosomes remained

stable within the cell cytoplasm of infected cells.

Once illuminated with either a cold white light or a laser beam, the consequent disruption caused the ODNs to become un-bound from the VP22. Once free, the ODNs were able to target their mRNA corresponding sequences – in this case, messenger RNA from the c-raf kinase gene.

Melanoma cell growth inhibited

In the case of melanoma cells, the researchers reported that when the cells were illuminated, growth was inhibited by up to 60%; without illumination, there was no effect on cell proliferation.

In their paper, the researchers commented that "the exact mechanism of light induced ODN release is poorly understood, but it requires that fluochrome be covalently linked either to the VP22 protein or to the ODN. It is assumed that thermal effects resulting from the absorbance of light by the fluochrome may cause the vectosome disruption and the ODN release throughout the cell".

A further objective of the research study was to characterise the mechanism of vectosome internalisation into cells by careful analysis using electron microscopy. The study found that "once within the cell cytoplasm, the vectosomes form well delineated endosome like vesicles without any apparent effect on the cell structure or

function.

When illuminated, disruption of the vectosome membranes occurred and was accompanied by the release of small dense particles migrating from the cytoplasm to the cell nuclei. These observations along with the high infection rate of VP22 indicate that the use of specifically designed vectosomes may be a safe alternative to the use of cationic lipids for infection of genes. Cationic lipid use is limited by their potential toxicity and their reduced efficacy in the presence of serum. Infection with VP22 on the other hand is not affected by serum and can be used *in vivo*".

Effective in vivo

The researchers additionally conducted tests of VP22/ODN complexes by intravitreal injections of up to 5 microlitres of vectosome preparation into the right eye of six rats using a 30-gauge needle, 2 millimetres posterior to the limbus. Examination of fluorescence microphotographs of retinal sections 24 hours after the injection showed a "pattern of vectosome localisation within the retinal cell layers consistent with a transretinal migration along the retinal Müller glial cells."

From these observations, the researchers concluded that light activated ODNs can be used as efficiently in cultured cells as in ocular tissues *in vivo*.