on normal blood cells. If those experiments go well, JS-K could look into a bright future. 'JS-K could possibly be used as a single agent, but I think it would also be a great drug to use in combination with other agents. There is nothing that we have currently available that works through that mechanism,' says Weinberg. Shami is already planning studies to combine JS-K with other standard chemotherapeutic agents to see if they can induce synergy.

The preclinical development of JS-K is also supported by the NCI. It has included the compound in its Rapid Access to Invervention Development (RAID) programme, which is aimed at speeding up the preclinical development of promising drug candidates. 'They have screened JS-K against a panel of 60 different cancer cell lines; it actually has a broader spectrum of activity than what we found it is active in,' hints Shami.

He and Keefer are now also working on optimizing the molecule. 'It is an interesting lead,' says Shami. 'It may not be the end product – we may develop a better compound still. But I think this would be a new class of chemotherapeutic agents.'

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Nanodevices hold promise for gene therapy

Hillary E. Sussman, freelance writer

The integration of nanotechnology with biology and medicine has been limited thus far to using bioconjugated nanoparticles, such as nanogold or quantum dots, for subcellular detection and tracking of biomolecules or as vessels for delivering drugs, such as antisense oligonucleotides [1–3].

Now, Tatjana Paunesku and Gayle Woloschak at Northwestern University, Chicago (http://www.northwestern.edu) and their colleagues at Argonne National Laboratory (http://www.anl.gov) have developed nanodevices that are able to target, bind and cleave DNA [4]. This novel approach to intracellular manipulation could become the next big thing in gene therapy – or the smallest!

Shedding light on TiO_2

The nanodevice is a chemical–biological hybrid composed of oligonucleotide DNA covalently attached to titanium dioxide (TiO_2) nanoparticles that are only 4.3 nm in size. The nanoparticle exhibits distinctive physical and chemical properties, because of the nature of TiO_2 as a wide-bandgap semiconductor and the increased surface area, compared to bulk TiO_2. 'Corner defects' make the nanoparticle more reactive and enable a stable covalent bond to be formed between it and dopamine end-labeled oligonucleotide DNA.

TiO_2 is also a photocatalyst: when illuminated, the absorption of photons with energy greater than it's bandgap (3.2 eV) results in excitation of electrons from the valence band to the conduction band, generating an electron hole pair, which acts like a positively charged particle. This translates into a unique photoinduced endonuclease activity when these electropositive holes are transferred along the DNA bound to the TiO_2 nanoparticle. According to Paunesku, the cumulative effects of repeated oxidation by these electropositive holes cause a double-strand break of the DNA, cleaving it from the nanoparticle.

Double-stranded breakthrough

'This powerful approach opens up future possibilities in medical biotechnology where different biomolecule-TiO_2 nanocomposites can be used in gene therapy,' says pharmacologist Dusica Maysinger at McGill University (http://www.mcgill.ca). In light of the fact that other biomolecules, such as RNA, peptides and sugars, can also be attached to TiO_2 nanoparticles, the possibilities are endless (see Fig.1). Woloschak, Professor of Radiology, envisions the nanodevices as multifunctional. For example, 'One thing we might want to do is attach one molecule of DNA or a peptide that could target it to a particular cellular site, and another peptide that can carry out an effector function,' she says.

The nanodevice can be used as a simple alternative to current methods of drug delivery, where 'you're just dumping DNA into the bag of the cell and hoping it's going to find its match,'

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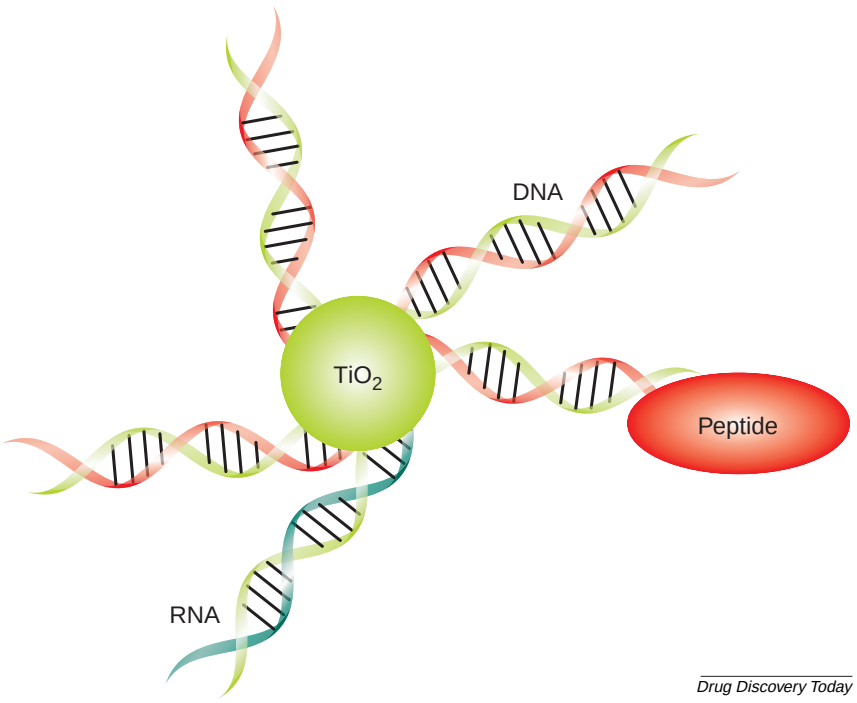

Figure 1. DNA, RNA and/or peptides covalently attached to TiO_2 nanoparticles could become new tools for gene therapy. Figure courtesy of Tatjana Paunesku, research Assistant Professor of Radiology at the Feinberg School of Medicine, Northwestern University, Chicago (http://www.northwestern.edu).

says Woloschak. According to her, antisense genes attached to the TiO_2 nanoparticle can be delivered to a particular intracellular site and, when illuminated, the antisense genes or the drugs will 'fall off in the right location'. And in the long run, because ionizing radiation also seems to photoactivate the nanodevice, 'the delivery of gene therapy or drugs to an intracellular target with the purpose of killing the cell could be done in combination with radiotherapy' in cancer patients.

Nanosurgery

'The impact of this approach has yet to be determined, but it could be far reaching,' says Eugene Cooper, Executive Vice President for Research and Development at Elan Corporation (http://www.elan.com). So far reaching that, in addition to simple drug delivery, these nanodevices might one day be used to perform nanoscale surgeries to repair a damaged gene.

Cells routinely repair double-strand breaks by excising damaged DNA and patching in DNA with sequence homology. In theory, attaching DNA repair machinery to the TiO_2 nanoparticle, such as a recombinase, in addition to an oligonucleotide used for recognition, would enable cleavage of mutated DNA via activation of the nanodevice with low-dose radiation equivalent to a diagnostic X-ray. Paunesku theorizes that it would increase the likelihood of having 'a good gene' patched in by attaching one with adequate border homology to the same nanoparticle that cleaves out the mutated gene. Woloschak adds, 'Similar things are being done now when people make transgenic mice but the efficiency is really poor and the process is random. We think that by using the titanium dioxide we can improve the efficiency and decrease the randomness of the system, by having everything attached to one scaffold.'

Just the beginning

At present, one of the limitations impeding gene therapy efforts is the difficulty of specifically targeting particular cells and subcellular locations. 'One of the biggest hurdles to solving the problems of intracellular manipulations by nanotechnology is the difficulty of introducing adequately controlled genetic material into the nucleus,' says Maysinger. So, the fact that the TiO_2-DNA nanodevices can be transfected into the nuclei of cells *in vitro* represents a challenge met. And by attaching a '50–60 base pair sequence [to the TiO_2 nanoparticle] that will take it only to one genomic location inside a mammalian cell,' Woloschak states, 'we may be able to get very specific cleavage. That really hasn't been achieved, yet.'

Chemist Dai-Wen Pang at Wuhan University in China (http://www.whu.cn) says, 'The use of TiO_2-oligonucleotide nanocomposites for gene therapy is really a unique concept…but it has a long way to go for practical use in gene therapy.'

'Much more work has to be done to prove their usefulness *in vivo*,' notes Maysinger. The researchers at Northwestern are first 'trying to get these things into mitochondria…to see what the range is in the cell,' and also testing the ability of the nanodevice to act as an alternative to RNA inhibitors by cleaving specific RNAs and interfering with RNA synthesis in cancer cells *in vitro*. But they agree that demonstrating the unique properties of the nanodevice in an *in vivo* model would be very important.

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