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Adverse effects of gene therapy

Hero or villain?

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Gene therapy is all about curing sick people. However, as the Jesse Gelsinger case and the recent leukemia case in the SCID trial tragically illustrated, in certain circumstances it can do more harm than good. Now David Dichek and his collaborators, in the course of their attempts to develop a treatment for thrombosis, have recorded another instance of gene therapy turning out to be more villain than hero.[1]

Atherosclerosis is a chronic disease that affects large and medium-sized arteries. Muscle cells, macrophages, cholesterol, connective tissue and calcium accumulate in clumps (lesions) in the inner layer of arteries (intima) of those affected.[2] The disease thus reduces blood flow, causing serious problems in the organs that depend on the arteries affected, especially in the heart, brain and extremities. The worst effects of the disease often eventuate when a blood clot (thrombosis) forms on top of an advanced lesion, sometimes as a result of it being ruptured, and further blocks an artery.

Thus, atherosclerosis requires both long-term therapy in order to reduce the effects of lesion burden and reduced blood flow, and acute treatments to dissolve or prevent thrombosis. The need for these therapies has increased during the last decade because cardiologists, vascular surgeons and radiologists are increasingly using several invasive vascular procedures that can cause thrombosis. These procedures include angioplasty, stent placement, vascular graft surgery, endarterectomias and prosthesis operations.[3]

Several groups have been working on gene therapy approaches to prevent thrombosis.[3] In the new study Dichek and his colleagues transferred genes for an enzyme used to treat thrombosis (urokinase-type plasminogen activator, uPA) into rabbit carotid arteries. As expected, they showed increased uPA expression (7–10-fold) in the arteries. However, to their great surprise, the increased uPA activity caused the arteries to constrict. One week after the gene transfer the arteries had major constrictions. Four weeks later the arteries had a 70% larger inner layer than control arteries.

These data indicate that elevated uPA expression promotes artherosclerosis. Importantly, this result suggests that increasing uPA activity in arteries with gene therapy (or any other means) would actually make things worse when treating acute complications of cardiovascular diseases.

Further work will be needed if we are to understand why elevated uPA expression promotes atherosclerosis. This is just one of many questions that the new study raises. For example, peak uPA expression occurred 3–7 days after the gene transfer and had returned to normal after 2 weeks, so why did the arteries not thicken until 4 weeks after the transfer? The role the uPA receptor plays in the process also needs to be investigated.

It is debatable whether the adverse effects of uPA gene transfer in rabbit arteries would also occur in humans suffering from atherosclerosis. There are a couple of key differences between the two situations. Firstly, the results from rabbit arteries were gathered over a time frame of weeks, whereas human atherosclerosis develops over decades. Secondly, in the rabbit arteries adenoviral expression occurred mostly in the endothelium whereas, in advanced human lesions uPA expression occurs mostly in macrophages. Thus, in future we need to look at the effects of increasing uPA activity in lesion macrophages.

It is not all bad news from the Dichek group's study. The study provides a great example of how an *in vivo* gene transfer study should be conducted. The authors looked at the effects of increasing vector dose (dose–response), as well as how the effects of the gene transfer changed with time (time–response). They also measured activity of the transferred gene from the tissues into which it was transferred. These measures should be taken in all *in vivo* gene therapy studies, to interpret properly the outcome of the experiments. Their results also show that local vascular gene therapy with adenoviral vectors is feasible and leads to easily measurable effects.

These new results show that in future there may still be a few surprises in store when studying local gene transfer in animal models. This new paper and similar recent ones show the power of such studies. Even the most advanced transgenic or knockout mouse experiments may be misleading compared to looking at the effects of therapeutic genes in a local environment. Chronic overexpression of a given gene or a knockout in a specific tissue type may still have too broad and long-lasting effects so that local acute changes are not detectable in such experiments.

Recent work has shown that, like uPA gene transfer, vascular endothelial growth factor (VEGF) can also promote atherosclerosis in animal models.[4] However, interestingly, in human studies there are no signs of worsening of atherosclerosis after local VEGF gene therapy.[5] The lesson to keep in mind when considering these new data is that we need to be very cautious about extrapolating results from animal models to humans. ■

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Vector integration

Pest not guest

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Retroviral integration into host cell chromosomes was once thought to be random, but new evidence suggests that human immunodeficiency virus preferentially enters the neighborhood of activated genes. This finding could have major implications for the use of retroviruses as gene therapy vectors.

Retroviruses are RNA viruses, but replicate through a DNA proviral genome intermediate. This intermediate is integrated into the host cell chromosomes and it is from here that transmissible RNA genomes are subsequently transcribed. The integrated provirus may be silenced in the host cell genome and vertically transmitted if it enters the germ line.

We now know that more than 40% of the human genome consists of retrovirus-like transposable elements, a feature likely to be common to all mammalian species.[1] Pathogenic retroviruses, such as human immunodeficiency virus (HIV), integrate into host cell chromosomes and produce large