

Adverse effects of gene therapy

Hero or villain?

S Ylä-Herttua

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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, endarterectomies 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 atherosclerosis. 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. ■

Seppo Ylä-Herttua is at the A.I. Virtanen Institute and the Department of Medicine, University of Kuopio, Kuopio 70210, Finland.

e-mail: seppo.ylaherttua@uku.fi

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Vector integration

Pest not guest

L-J Chang

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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

amounts of progeny virus that spread horizontally. Understanding the process of retroviral integration may help explain how the human genome has evolved and how retroviral infection may cause genomic damage. It will further help us assess potential risks of gene therapy using retroviral vectors.

In a recent study, published in *Cell*, Frederic Bushman and co-workers² mapped HIV type 1 (HIV-1) integration sites in the human genome. Bushman's group sequenced 524 integration junctions between viral and host DNA in an HIV-infected human cell line, and then located these in the human genome sequence. They then looked at the cDNA expression profile after viral infection to compare the locations of genes that are active after infection with the viral integration sites.

An impressive 67–86% of HIV-1 integration sites were found in transcribed regions of the human genome. Moreover, gene activity surrounding the integration hot spots increased by 2–3 fold in general after infection, suggesting a close association of viral and host gene expression.

As a control, the researchers also prepared naked DNA from the same T cell line as target DNA and HIV-1 preintegration complexes (PICs) from infected T cells so they could analyze *in vitro* integration events. They found that roughly 35% of the *in vitro* integration sites were in transcribed regions of the human genome. Since ~33% of the human genome is present in transcription units anyway, this is about what you would expect if integration was random.

The new data confirm previous observations that retroviral integration favors transcribed genes.^{3,4} However, importantly, their analysis of host gene expression affected by HIV-1 infection showed, for the first time, that HIV-1 targets genes that are activated both before and after infection.

The pattern of HIV-1 integration clearly differs from those of the vast majority of human endogenous retrovirus (HERV) sequences. HERV sequences represent up to 1% of the human genome, but are located mostly outside of gene clusters or exons and thus probably do not affect gene function.⁵ By contrast, these new findings clearly put the integrated HIV-1 provirus in the "pest" sequence category because targeting ac-

tively transcribed genes is likely to interfere with cellular function. Clinical evidence has now been reported. Following the successful treatment of X-linked severe combined immunodeficiency (SCID), one of the 10 children who had received murine leukemia virus (MLV) vector modified bone marrow cells has developed acute lymphoblastic leukemia, apparently caused by integration interruption of a possible oncogene located in chromosome 11.⁶

It is still not clear how HIV-1 PICs find the hot spots that are preferentially associated with the polII transcriptional units. Cellular components associated with chromatin structure and transcriptional machinery may affect retroviral integration directly or indirectly. For example, both MLV and HIV-1 favor sites of active chromatin assembly and DNA looping or bending.^{7–9} This raises the possibility that physiological conditions that change chromatin structure and transcription profile (eg stress and hormone signaling) could affect HIV-1 infection and integration, and consequently disease progression.

Retroviral DNA contains two viral polyadenylation signals. When integrated in the same orientation as the targeted host gene, the viral polyadenylation signals may interrupt host RNA transcription and processing. HERV elements present in intragenic regions in the human genome are preferentially inserted in the opposite orientation from that of the host gene. However, the HIV-1 integration sites in the *Cell* study showed no orientation preference relative to that of the targeted genes.² The polyadenylation signal of retrovirus is known to be leaky and allows frequent transcriptional readthrough. However, the HIV-1 polyadenylation signal is not as leaky as that of the MLV.¹⁰ Thus, HIV-1 integration into active genes may be more detrimental to the host cells than MLV. This is consistent with the observation that a high multiplicity of HIV-1 infection often causes cytotoxicity and apoptosis of the target cells.

In yeast, retrotransposons such as Ty elements shuttle freely in the host cell genome, but as respectful 'guests' they target specific sites with high precision, such as upstream of the polIII promoters or nonfunctional regions in the yeast genome.¹¹ This strategy prevents host genes from being interrupted. Regardless of the

'guest' or 'pest' nature, both Ty elements and HIV-1 must have evolved mechanisms to choose their preferred sites in the host chromosomes. The interaction of retroviral PICs with host cell factors, such as chromatin components or polII and polIII transcription factors, may help define the genome 'hot spots' for the transposable DNA.

It is still unclear which parts of the PICs interact with cellular factors to direct retroviral integration. Understanding the mechanisms by which retroviruses and retrotransposons are specifically integrated will shed light on additional strategies of HIV-1 intervention, as well as assist future development of targeted retroviral gene delivery systems.

When HIV-1-based vectors are used for gene therapy, a high multiplicity of infection is always associated with increased cell death. The new work by Bushman and his colleagues suggests that preferential integration of the vectors into hot spots surrounding active host genes could explain this. It might be possible to modify vector components and cell growth conditions to channel PICs into desired chromosomal locations. The ability to target retroviral integration will significantly improve safety and specificity of retroviral vectors for future gene therapy applications. ■

Lung-ji Chang is in the Department of Molecular Genetics and Microbiology, Powell Gene Therapy Center and McKnight Brain Institute, University of Florida, Gainesville, Florida, USA.

e-mail: lchang@mgm.ufl.edu

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serotypes from chimps that may provide the solution to this tricky problem.¹

AAVs seemed to be the ideal solution for problems that adenoviral vectors have in delivering long-term expression of transgenes in the target region. These problems result from the immune and inflammatory response adenoviral vectors provoke, which usually leads to rapid elimination of the transduced cells.^{2–4}

Unlike adenoviruses, AAVs are well tolerated and do not cause a strong innate end response or cytotoxic T cell response. In addition, transgenes delivered with AAVs tend to be expressed for longer than adenoviral-delivered genes. However, AAVs still do provoke an antibody re-

Adeno-associated viruses

Monkey see, monkey do

JD Mountz

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Like many other gene therapy vectors, effectively transfecting target cells without provoking an antibody response that limits readministration has

often proved to be a bridge too far for adeno-associated viruses (AAVs). Now James Wilson's group at the University of Pennsylvania has isolated two new AAV