Parachuting in the epigenome: the biology of gene vector insertion profiles in the context of clinical trials

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Retroviral pre-integration complexes (PICs) provide a most efficient mechanism to integrate foreign DNA into cellular chromatin. This has made retrovirus-based vectors a preferred tool for gene delivery in therapeutic settings where the chromosomal integration of a recombinant expression cassette that encodes a protein of interest can lead to a long-lasting correction of monogenetic diseases (so-called gene addition strategy). However, the efficiency of retroviral gene addition comes at the expense of a lack of precision in the choice of the integration site. Insertional mutagenesis with potential activation of proto-oncogenes as a first hit in a multi-step scenario of cancer development thus represents one of the major hurdles to a more widespread exploration of gene-based treatments (Kustikova et al., 2010). To date, four clinical trials were reported to be associated with severe adverse reactions induced by insertional mutagenesis in haematopoietic stem and progenitor cells (HSC/P); two targeting the X-linked form of severe combined immunodeficiency (SCID-X1), one targeting chronic granulomatous disease, and most recently another trial exploring gene therapy for the Wiskott–Aldrich Syndrome. In contrast, in the SCID caused by mutations in the gene encoding the metabolic enzyme adenosine deaminase (ADA), retroviral gene addition so far has been free of such complications. Furthermore, numerous trials using similar gene vectors to transfer genes into mature T cells have not been complicated by clonal outgrowth. This explains the great interest in a deeper understanding of retroviral vector–host interactions in the therapeutic setting of SCID-ADA.

ADA is involved in purine metabolism and detoxification, and its deficiency thus affects many organs with lymphoid cells being the least resistant. Enzyme replacement therapy (ERT) using repeated infusions of bovine ADA only leads to a partial recovery of immune function, which can prevent infectious complications, but typically does not result in a complete normalization of the immune system and does not fully prevent non-lymphatic organ toxicity of purine metabolites. Bone marrow transplantation from a fully human leukocyte antigen (HLA)-matched donor has a relatively good prognosis especially if performed in the first year of life. For affected children lacking suitable donors and access to ERT, gene therapy has been established as a promising alternative, now with orphan drug status in the EU. To date, with more than 10 years follow-up, encouraging data have been reported from a study in Milan, Italy, where gene vectors based on murine leukaemia virus (MLV) were used to place an intact copy of ADA into autologous HSC/P, thus expressing ADA under control of the original retroviral regulatory elements residing in the long terminal repeats (LTRs; Aiuti et al., 2009). Before targeting HSC/P, first clinical trials were performed to explore the potential to express ADA after retroviral gene transfer into mature T cells. However, given the systemic nature of this metabolic disorder and the need for life-long regeneration of lymphocytes, HSC/P were considered the more relevant target. Molecular clonality studies of haematopoietic reconstitution in gene therapy of SCID-ADA may thus provide substantial biological information on the speed and kinetics of lymphatic cell regeneration, the clonal complexity of haematopoiesis at the systemic level and the interplay of vector and host features on the cellular level.

As a result of the stepwise improvements of clinical gene therapy protocols, Aiuti et al in Milan not only have presented encouraging clinical data with long-term correction of immunity and restoration of normal development in SCID-ADA children (Aiuti et al., 2009); based on the necessity of long-term follow-up with detailed monitoring of vector insertion sites, they also have access to a unique resource of gene-modified cell samples. In
this issue of EMBO Molecular Medicine, they present a detailed analysis of retroviral vector integration patterns, comparing samples archived pre- and post-transplantation in the two different gene therapy approaches targeting either T cells or HSC/P (Biasco et al, 2011). This comparison is of substantial interest as (1) not only clinical data but also preclinical 'high-risk' models suggest that the probability of malignant cell transformation by insertional oncogene activation is far higher in HSC/P than in mature T cells (Kustikova et al, 2010); and (2) chromatin remodelling is known to precede reorganization of gene expression during differentiation (Shen & Orkin, 2009) and affects both retroviral integration site selection and retroviral gene expression. Furthermore, understanding the interplay of chromatin remodelling and retroviral genomes connects the field of gene therapy with investigations of viral dynamics upon infection with the human immunodeficiency virus (HIV). In gene therapy, chromatin interaction may be decisive for the insertional risk profile and the likelihood of long-term expression of the therapeutic gene; in the context of HIV, chromatin modifications have been identified as a major mechanism that establishes and controls viral latency, thus representing a major obstacle towards the holy grail of HIV eradication (Colin & Van Lint, 2009).

By revealing the cell-specific expression profile of genes 'hosting' vector integration events, the new study by Biasco et al underlines the substantial impact of transcriptional and chromatin features for the distribution of integration sites in the clinical context. Furthermore, overlaying their set of >4000 identified insertion events on previously established datasets of histone methylation profiles in human HSC/P and T cells (reviewed by Shen & Orkin, 2009), they found a higher frequency of integrations associated with open chromatin marks.

Figure 1. The retroviral PIC, based on MLV in the context of the clinical trial for SCID-ADA, is composed of the double-stranded vector genome containing the ADA gene flanked by LTR and retroviral proteins including the integrase. Other retroviral and host proteins decorate this complex, attracted through DNA–protein and protein–protein interactions. In the search for a suitable landing spot in the host genome, cell-specific epigenetic modifications play a major role. Histone marks associated with repressed genes (H3K27me3, among others) are disfavoured as insertion sites (red indicates DNA with CpG methylation, pins indicate additional histone modifications), whereas marks associated with active genes are preferred (H3K4me1, H3K27me1, H3K4me3). Furthermore, host proteins attracted to regulatory elements surrounding the transcriptional start site (TSS, +1) and DNAse I hypersensitive sites (DNAse I HSS) are likely to play a major role in attracting MLV PICs.

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(H3K4me1, H3K27me1 as marks of gene activation; H3K4me3 as mark of transcriptional start sites) and a negative correlation with a typical repressive histone mark (trimethylated histone H3K27me3; Fig 1). This global pattern was confirmed when selecting two cell-specific hot spots for a more detailed portrait of the epigenome (the T cell receptor locus, TCRA, for T cells and a clinically relevant proto-oncogene locus, LM02, for HSC/P). Their study highlights that transcriptional profiling should best be coupled with epigenetic landscape analyses to understand how the nuclear environment, which starts to rearrange chromatin long before the definitive onset of differentiation-specific gene expression (Shen & Orkin, 2009), offers landing platforms for the retroviral PIC. As numerous additional histone decorations have been described (various forms of ubiquitination, phosphorylation, SUMOylation, ADP-ribosylation acetylation and methylation) and DNA modifications are also involved in epigenetic decorations (Colin & Van Lint, 2009; Shen & Orkin, 2009), it can be expected that future studies, especially when coupled with the ever increasing power of high-throughput insertion site sequencing, will reach an even higher resolution and even more stringent correlation of the epigenomic landscape and the retroviral integrome. A paradigmatic study revealing the potential of this approach to genetically mark regulatory sites in primitive haematopoietic cells has just been published (Cattoglio et al, 2010).

While the major differences in the epigenetic landing areas for incoming PICs may contribute to the lower risk of retroviral gene transfer in T cells compared to HSC/P, this mechanism is less likely to explain the increased risk profile observed in gene therapy for SCID-X1. Potential signalling alterations induced by over-expression of the therapeutic gene remain controversial (in SCID-X1, a signalling receptor subunit, IL2RG, has to be expressed whereas in SCID-ADA, a metabolic gene is encoded from the vector). Other disease-associated risk factors such as replication stress and genetic instability currently appear more likely to play an important role in the differential risk of insertional complications (Kustikova et al, 2010). While redesigned vectors have the potential to reduce the risk of insertional mutagenesis, the exploitation of epigenetic mechanisms also has a lot to offer in the context of genetic cell modification. For future gene therapy protocols targeting primitive stem cell populations, it is important to consider that the procedures contributing to cell isolation and culture may have an impact on the epigenome and thus on the insertional repertoire. Furthermore, epigenetic treatment interventions may have a role to play to reactivate silenced transgenes, as attempted in the eradication of latent HIV reservoirs.

If the epigenome can be modified, can we also manipulate the retroviral PIC to access other genomic regions? The PIC, being composed of retroviral proteins such as integrase with remnants of the capsid and the proviral genome in the form of double-stranded DNA, is not a completely passive structure that lands in accessible genome loci like an unconscious parachutist. While the proviral DNA, depending on its sequence composition, may attract host proteins including transcription factors and chromatin remodelling proteins already prior to integration, the retroviral protein components of the PIC can act as additional attractors of host proteins (Fig 1). In the context of HIV, the cellular protein p75/LEDGF has been shown to play a major role in the preference of the PIC to land in actively transcribed regions of the genome. In the context of MLV, the exact co-factors that guide the PIC in the vicinity of transcriptional start sites and other sites of active chromatin remain to be identified. The modification of PIC components may thus allow to direct gene vector integration preferences away from potentially problematic sites, and ultimately into safe genomic harbours. In this context, the study of Biasco et al beautifully demonstrates that PICs have to follow social rules: they cannot reckon without their host.

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