

Commentary

Recent translational research: Oncogene discovery by insertional mutagenesis gets a new boost

John Hilkens

Division of Tumor Biology, The Netherlands Cancer Institute, Amsterdam, The Netherlands

Corresponding author: John Hilkens, j.hilkens@nki.nl

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Abstract

Knowledge of the genes and genetic pathways involved in oncogenesis is essential if we are to identify novel targets for cancer therapy. Insertional mutagenesis in mouse models is among the most efficient tools to detect novel cancer genes. Retrovirus-mediated insertional mutagenesis received a tremendous boost by the availability of the mouse genome sequence and new PCR methods. Application of such advances were limited to lymphomagenesis but are now also being applied to mammary tumourigenesis. Novel transposons that allow insertional mutagenesis studies to be conducted in tumors of any mouse tissue may give cancer gene discovery a further boost.

Introduction

Oncogenic transformation of a normal cell is a multistep process that requires activation or inactivation, usually by mutagenic events, of several key genes controlling various essential cellular pathways that are involved in growth and development. An understanding of which pathways and genes are involved is essential to the development of novel therapeutic strategies. Although a large number of genes involved in breast and other cancers has been discovered, the picture is far from complete. Retroviral insertional mutagenesis (IM) in mouse models is among the most efficient tools to uncover cancer genes. The most important advantage of IM over chemical and radiation induced mutagenesis is that the mutagen is also a molecular tag, facilitating the identification of the mutated locus. Because proviral integration is essentially random with some bias for transcriptionally active domains, retroviral insertions in the same genomic locus in multiple independent tumours mark a so-called common insertion site (CIS), which is likely to contain a gene involved in tumourigenesis.

IM was first described in 1981 by Hayward and coworkers [1] when those investigators, in an effort to elucidate the mechanism by which avian leukosis virus induces lymphomas, discovered that the virus targeted the cellular *Myc* gene. Shortly thereafter, Nusse and Varmus [2] identified *Wnt1* as a frequent

target of mouse mammary tumour virus (MMTV) using this approach. IM mediated by retroviruses is based on the integration of a DNA copy of retroviral RNA as a provirus into the cellular genome as part of the life cycle of the virus. The regulatory elements in the proviral long terminal repeats can then transcriptionally activate adjacent cellular genes. Proviral insertions that truncate cellular genes giving rise to dysfunctional or constitutively active proteins are also frequently found; for an extensive overview of retroviral IM, see the reports by Jonkers and Berns [3] and Mikkers and Berns [4].

Although these landmark studies were followed by several others, the number of oncogenes discovered in this way remained rather limited. This was mainly due to the rather laborious techniques, including Southern blotting, extensive cloning of DNA fragments and 'chromosome walking', needed to identify the affected gene. The availability of novel PCR technologies such as the splinkerette [5] and inverse PCR [6], but in particular the completion of the draft sequence of the mouse genome, permitted high-throughput screening strategies and caused a revival of the IM approach to gene discovery. Hundreds of novel candidate oncogenes involved in lymphomagenesis have recently been reported using Moloney murine leukaemia virus (MoMuLV) [5,7-10]. Despite these technological advances, no large-scale screening for CISs in other neoplasias was conducted until very recently. Here I review some of the recent papers that used new opportunities for gene discovery in brain and mammary tumourigenesis [8,11,12]. In addition, I discuss transposon-mediated IM in mice, which has now achieved a degree of maturity and in theory allows high-throughput cancer gene discovery studies in any tissue.

Insertional mutagenesis in mouse mammary tumours

Thus far, no more than 12 genes with proven oncogenicity have been found by MMTV tagging in mouse mammary

CIS = common insertion site; IM = insertional mutagenesis; MMTV = mouse mammary tumour virus; MoMuLV = Moloney murine leukaemia virus; PB = PiggyBac transposon; PCR = polymerase chain reaction; PDGF = platelet-derived growth factor; SB = Sleeping Beauty transposon.

Table 1

Oncogenes and candidate oncogenes detected by MMTV insertional mutagenesis in mouse mammary tumours

Gene	Gene product	Confirmed oncogenicity	Evidence for involvement in human breast cancer	Reference
<i>Fgf3/int2</i>	Fibroblast growth factor	Yes	No	[22]
<i>Fgf4</i>	Fibroblast growth factor	Yes	No	[23]
<i>Fgf8</i>	Fibroblast growth factor	Yes	Yes	[24]
<i>Fgf10</i>	Fibroblast growth factor	Yes	Yes	[11]
<i>Int-5/Int-H</i>	Aromatase	Yes	Yes	[25]
<i>Int6/Eif3e</i>	Translation initiation factor 3 subunit	Yes	No	[26]
<i>Int7</i>	Thrombospondin domain containing gene	No	No	[12]
<i>Int-41</i>	Unknown	No	No	[27]
<i>Map3k8/Tpl2</i>	Mitogen-activated protein kinase	Yes	Yes	[28]
<i>Notch1</i>	Receptor/transcription cofactor precursor	Yes	Yes	[29]
<i>Notch4/Int3</i>	Receptor/transcription cofactor precursor	Yes	No	[30]
<i>Wnt/int1</i>	Morphogen	Yes	No	[2]
<i>Wnt3</i>	Morphogen	Yes	No	[31]
<i>Wnt10b</i>	Morphogen	Yes	Yes	[32]

MMTV, mouse mammary tumour virus.

tumours (Table 1). One of these (*Int5*) may only be involved in hormone-dependent growth, whereas the oncogenic potential of two genes (*Int41* and the novel *Int7* gene [12]) remains to be established. Although not all of these genes are involved in human breast cancer, most of the pathways in which they act have been implicated in human disease. Remarkably, the list of confirmed oncogenes is mainly composed of *Wnt*, *Fgf* and *Notch* genes, which are involved in development of the embryonic mammary gland. *Int7* belongs to a novel class of oncogenes [12]. Although its oncogenicity is yet to be established, its discovery shows that MMTV-mediated IM still has the potential to identify new classes of oncogenes in mammary tumourigenesis. Also, the sky rocketing number of candidate oncogenes found in the high-throughput MoMuLV mediated IM studies in lymphomagenesis indicates that MMTV-mediated gene tagging in mammary tumourigenesis needs further exploration. An obvious question arising is whether proviral MoMuLV and MMTV may tag the same genes. Comparison of the loci tagged by MMTV in mammary tumours identified in our own recent semi-high-throughput study (Theodorou V, Boer M, Kimm M, Theelen W, Jonkers J, Hilkens J, unpublished data) with the loci tagged by MoMuLV in murine lymphomas deposited in the mouse Retrovirus Tagged Cancer Gene Database [13] suggests that the overlap is small. This indicates that the cancer-associated signal transduction pathways in mammary tumourigenesis and lymphomagenesis are at least partly different and warrants further high-throughput IM studies.

New technological developments

Insertional mutagenesis with modified retroviruses

Collaborating oncogenes (i.e. genes that act together in tumourigenesis) can also be identified by IM because they

are frequently coactivated in the same tumours. IM in oncogene-expressing transgenic mice is frequently used to identify oncogene collaboration more directly. This method was used to show, for example, that *Wnt* and *Fgf* genes collaborate in mammary tumourigenesis [14]. Recently, Johansson and coworkers [8] used another IM approach to seek collaborating genes. Based on the notion that autocrine stimulation of the PDGF receptors is a frequent event in glioblastoma development, they administered to mice an intracerebral injection of recombinant MoMuLV encoding platelet-derived growth factor (PDGF)- β (together with a wild-type MoMuLV helper virus). The recombinant virus not only predisposed these mice to gliomas, but MoMuLV also efficiently tagged oncogenes in the brain, potentially collaborating with PDGF- β . This experiment also shows that nonhaematopoietic cells can be predisposed in such a way that these cells accumulate sufficient insertions to develop into tumours before haematopoietic cells, which are the prime targets of MoMuLV, can do so. However, local virus injection may also have contributed to the absence of lymphomas in these animals.

The authors identified 66 loci that harbour potential brain tumour-associated oncogenes that collaborate with PDGF- β . In a subsequent report [15], it was shown that most of the common retroviral insertions indeed affected the predicted genes – evidence that recent high-throughput studies often lack. Some of these candidate oncogenes were previously not known to be involved in (brain) tumourigenesis.

Transposon-mediated cancer gene tagging

Until recently, retrovirally induced IM was not applied in solid tumours with the exception of mouse mammary tumours and

the brain tumours discussed above because of tissue tropism of the retroviruses. Transposon-mediated IM may be more widely applicable and is highly efficient, as has been shown in *Caenorhabditis elegans* and *Drosophila* [16,17]. DNA transposons are discrete DNA elements that are able to translocate within the cellular genome by excision and reinsertion mediated by a transposase, which can be encoded by the transposable DNA element or can be located elsewhere in the genome. No active DNA transposons have been identified in mammalian genomes. However, recently no fewer than three papers described artificial transposable elements designed for IM in the mouse. Dupuy [18] and Collier [19] and their coworkers used an adapted Sleeping Beauty (SB) transposon that was originally resurrected from evolutionary inactive elements in fish DNA, whereas Ding and colleagues [20] adapted PiggyBac (PB) derived from a DNA transposable element in a cabbage moth. Both are binary systems (i.e. the transposase and transposon are independently transmitted and can be maintained in different animals that can be interbred to mobilize the transposon). PB was shown to efficiently integrate and transpose randomly not only in cell lines but also in the mouse germ line. This transposon may thus be particularly useful for germ line IM. However, the low number of transpositions per generation or cell division may limit the use of PB for cancer gene discovery in its present form.

Dupuy [18] and Collier [19] and their coworkers have adapted the SB system more specifically for IM in somatic cells by providing the transposon with viral promoter and enhancer elements for gene activation, and splice donor, acceptor and polyadenylation sequences for induction of intragenic insertional mutations by gene trapping. SB efficiently mobilizes in somatic cells and reinserts in or close to genes, thereby modifying gene expression and/or interrupting coding sequences by similar mechanisms as employed by retroviruses. However, DNA transposons hop through the genome by a cut and paste mechanism and proliferate very slowly, which is inhibitory for efficient somatic mutagenesis. Dupuy [18] and Collier [19] and their coworkers solved this problem with SB to some extent by introducing large concatamers containing up to 300 copies of the transposon into the mouse genome. A shortcoming of the SB system used by Collier and coworkers [19] is its tendency to transpose to locations close to the excision site. This means that even after several rounds of transposition the majority of the transposons still tend to land on the same chromosome. The transposon density on the originating chromosome can be so high that the significance of the less frequently tagged loci may be doubted. Although mobilization of the Dupuy variant was more efficient, allowing the transposons to spread wider and to different chromosomes, this system suffered from a high incidence of embryonic lethality and a strong predisposition to haematopoietic tumours. Both issues may be resolved by the use of tissue-specific and/or inducible SB-based mutagenesis systems, in which tissue specificity and/or timing of transposase activity can be controlled.

Conclusion

Discovery of new genes with a causal role in cancer and delineation of the pathways through which these genes act form the basis for designing novel cancer treatments. Retroviral IM has already proven to be invaluable for this purpose and transposon-mediated IM will broaden further its application to malignancies other than lymphomas and mammary tumours. Because lymphoma genes tagged by transposons only partly overlap with genes targeted by retroviruses, both technologies may complement each other.

Exhaustive screening for IM screens will yield many genes with such a low contribution to oncogenesis that therapeutic targeting of these genes or gene products is ineffective. This may be particularly true for infrequently hit genes. Moreover, the availability of the Retrovirus Tagged Cancer Gene Database [13] in principle allows different studies to be combined, and it may be tempting to conclude that loci that are sporadically hit in separate studies are common, without consideration of statistics. However, the genomic window in which the insertions must be present for a statistically meaningful CIS will become very small as the number of analyzed insertions increases [5]. The size of such windows may even be overestimated, because both proviruses and transposons tend to land preferentially in transcriptionally active regions of the genome [21]. Furthermore, the affected gene may not always simply be the adjacent gene. This means that candidate cancer genes identified from IM screens should ultimately be validated in an oncogenicity study.

Another point to consider is that cancer genes may affect different tissues when they are mutated in mice and in humans; therefore, conclusions of experiments obtained with murine cancer genes should be carefully assessed before applying them to human cancer. Searching the available microarray expression data for over-expression of the studied mouse gene in human cancer may already provide a quick indication of the relevance of this gene for human cancer.

Extrapolating from the number of genes found by IM in lymphomas, we may expect a large number of novel candidate oncogenes to be discovered by IM in other tissues. In mammary carcinogenesis the novel genes will come from MMTV-mediated IM, and the list will probably be completed by transposon tagging. In other tumours the full benefit of transposon-mediated IM will be obtained. The next challenge will be to prove the true contributions of all these genes to oncogenesis, to find the pathways in which they act, and to exploit this knowledge for therapy.

Competing interests

The author(s) declare that they have no competing interests.

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