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Non-identical patterns of proviral insertions around host transcription units in lymphomas induced by different strains of murine leukemia virus

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Abstract

In a small sample of 57 retrovirus integration sites (RISs) isolated from 23 end-stage lymphomas induced in NMRI mice by the B-lymphotropic Akv wt or an enhancer mutant hereof, Akv1–99, we identified 14 novel RISs and defined 9 novel CISs (common insertion sites). Moreover, when comparing with RISs from tumors induced by the T-lymphomagenic SL3-3, we observed that SL3-3 targets RefSeq promoter regions with a significantly higher frequency than Akv/Akv1–99 and in an orientation-dependent way. Altogether, our results strongly emphasize the importance of host genetic background and virus type for retroviral insertion mutagenesis screens and suggest that different types of MLV may favor specific genomic regions and orientations in order exert optimal effect on target gene expression during lymphoma induction and development.

Keywords: Murine leukemia virus; SL3-3; Akv; Provirus; Common integration site (CIS); Retrovirus integration sites (RIS); Lymphomas; Insertional mutagenesis

Introduction

Tumor induction by the non-acute-transforming retroviruses is a multistep process in which insertional mutagenesis plays a fundamental role and where the effect of the individual integrated provirus will depend on the particular location of the insertion site relative to the targeted gene. The integrated provirus may affect the neighboring genes in a variety of ways by what have been described as promoter insertion, enhancer insertion, and/or truncation of a normal cellular gene (Rosenberg and Jolicoeur, 1997; Uren et al., 2005).

In the era of post-genome-sequence completion, retroviral insertional mutagenesis in mice has proven its significance as a potent instrument to identify candidate cancer genes, in particular, those related to diseases in the hematopoietic system (Erkeland et al., 2004; Hansen et al., 2000; Joosten et al., 2002; Kim et al., 2003; Li et al., 1999; Mikkers et al., 2002; Sorensen et al., 1996). Several independent screening studies have thus mapped the exact positions and orientations of hundreds of proviral insertions, and much of the published collection of miscellaneous retrovirus integration sites (RISs) has been organized in the Retroviral Tagged Cancer Gene Database (RTCGD; http://RTCGD.ncifcrf.gov; Akagi et al., 2004). For the present, this database (version mm6) contains more than 2200 insertions, which define more than 350 common integration sites (CISs).

Evidently, the strength of combining RISs from many different sources lies in the opportunity of discovering novel rare CISs and improving the resolution of already known CISs. However, this also precludes detailed and faithful comparison studies between different model systems since several parameters will differ between the various studies; parameters of which the most influencing ones would be different host genetic backgrounds, different virus strains, and different PCR-based strategies for tag identification. As an example, we have in a previous study examined the integration site pattern around the *Fos/Jdp2/Batf* locus in tumors induced by SL3-3 or Akv murine leukemia viruses (MLVs) in NMRI or SWR genetic background (Rasmussen et al., 2005), and we noted a clear difference from the pattern within the

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same locus observed by Hwang et al. (2002), whose studies were based upon Moloney MLV-induced tumors in p27+/+ or p27−/− C57/B6J×129/Sv hybrid mice. Due to usage of different mouse strains, different viruses, and different PCR-based methods, it was not possible to determine whether host genetic composition and/or inherent viral features were the main contributor to the observed differences. To clarify such issues, equivalent experimental setups are needed.

Here, we report on the analysis of provirus integration sites from three comparable studies, where the mouse strain and the PCR method for tag identification are unchanged. We observe that in end-stage lymphomas the T-lymphomagenic SL3-3 MLV is found much more frequently in promoter regions than the B-lymphomagenic Akv and Akv1–99 MLVs. Likewise, we see a significant lack of Akv1–99 insertions downstream of the target gene compared to Akv wt. Due to the comparable experimental setups, the observed differences in integration site pattern in end-stage tumors can be associated with virus characteristics rather than mouse genetic background or PCR strategy. Moreover, by using this particular combination of mouse strain, virus types, and PCR method, we identify 14 novel RISs and define 9 novel CISs.

Results and discussion

Integration site analyses of Akv- and Akv1–99-induced tumors

Akv1–99 is an ecotropic MLV derived from Akv MLV by deletion of one copy of the 2 × 99-bp transcriptional enhancer in the proviral LTR (Fig. 1). Both viruses induce B-cell lymphomas with nearly 100% incidence in randomly bred NMRI mice and with a mean latency period of about 12 months (Lovmand et al., 1998). By a simple two-step PCR method, which has previously been described as an efficient technique for the isolation and sequencing of provirus–host junctions (Martin-Hernandez et al., 2001; Sorensen et al.,1993, 1996), we have from 23 Akv-wt- and Akv1–99-induced tumors from an earlier study (Lovmand et al., 1998) amplified a total of 57 proviral flanking sequences, representing 28 tags from 10 Akv-wt-induced tumors and 29 tags from 13 Akv1–99-induced tumors. Of these, 24 and 26 tags, respectively, could be located within ±100 kb of a RefSeq (Maglott et al., 2000; Pruitt and Maglott, 2001) (Fig. 2, upper panel). Since the RTCG database already contains about 2300 RISs, it was not foreseen that we from such a small sample were able to identify 14 novel RISs (Table 1) and furthermore define 9 novel CISs (Table 1). Some of the identified RISs may be "passenger integrations" that by chance have been drawn into end-stage tumor cells, but play no role in the oncogenic process (a feature that may as well apply to RISs already included in the RTCGD). However, since CISs define regions or genes that are targeted by two or more viruses in independent experiments, these regions most likely are of biological relevance and do contribute to induction or progression of tumor development. Thus, altogether our findings strongly emphasize the importance of the experimental model system.

As previously published, the N-*ras*/*unr* locus defines a CIS within the Akv1–99 setting (Martin-Hernandez et al., 2001) since two tags target this chromosomal region, and, in addition, we have defined the *Pvt*-1 locus (downstream of c-*myc*, a position classified as c-*myc* RIS within the RTCGD) as a CIS within the Akv wt setting since two tags out of 28 could be addressed to this locus (see Table 1).

Distinct regions of the target gene are hit by Akv wt, Akv1–99, and SL3-3

The collection of Akv wt and Akv1–99 sequence tags isolated from comparable experimental settings allowed us to evaluate if a general difference in integration site position relative to the targeted gene could be detected. Besides, we were able to include in this analysis sequence tags isolated from SL3-3-induced tumors from one of our early studies (Sorensen et al., 1996) since those tumors were induced in the same host genetic background (randomly bred NMRI mice) and the tags were isolated by means of the exact same PCR method. In contrast to Akv (and Akv1–99), SL3-3 induces primarily T-cell lymphomas with an average latency period of about 3 months in ran-

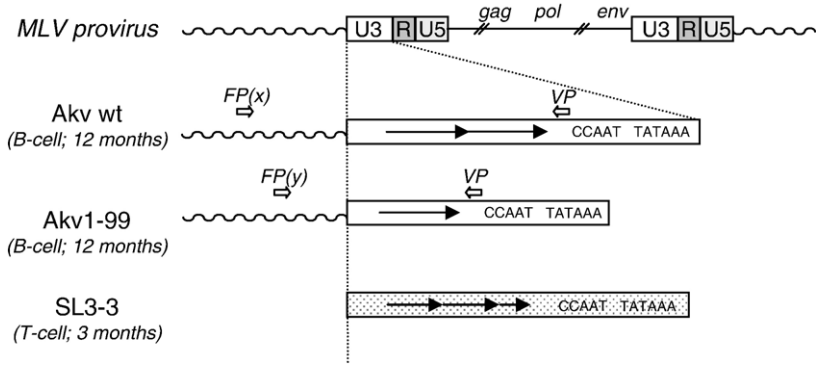

Fig. 1. Genomic structures of Akv, Akv1–99, and SL3-3 proviruses. Akv MLV contains a 99 bp tandem repeat in the U3 enhancer region, while Akv1–99 MLV contains only one copy of this 99 bp sequence. The SL3-3 LTR contains different U3 enhancer repeats consisting of 2 1/2 repeats (2 × 72 (=34 + 38) bp + 34 bp). Below each virus name, tumor phenotype and average latency period in randomly bred NMRI mice are given in parentheses. Curved lines indicate flanking cellular sequences. FP = flanking primer, which is constructed and specific for each integration sites. VP = virus-specific primer.