Title: RAG-dependent recombination at cryptic RSSs within *TEL-AML1* t(12;21)(p13;q22) chromosomal translocation region

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Abstract

The recombination activating gene (RAG) is a lymphoid-specific endonuclease involved in the V(D)J recombination. It has long been proposed that mis-targeting of RAG proteins is one of the factors contributing to lymphoid chromosomal translocation bearing authentic recombination signal sequences (RSSs) in *immunoglobulin* (*Ig*) and *T cell receptor* (*TCR*) gene loci or cryptic RSSs (cRSSs). However, it is unclear whether primary sequence-dependent targeting mistake involved in the chromosomal translocation bearing no *Ig*/*TCR* gene loci is mediated by RAG proteins. Using an extrachromosomal recombination assay, we found RAG-dependent recombination in the regions dense in breakpoints within *TEL* and *AML1* gene loci related to acute lymphoid leukemia-associated t(12;21)(p13;q22) chromosomal translocation. Sequence analyses revealed several heptamer-like sequences located in the vicinity of RAG-dependent recombination sites. By chromatin immunoprecipitation (ChIP) and ligation-mediated PCR (LM-PCR) assays, we have shown that RAG proteins bind to and cleave the *TEL* translocation region dense in breakpoints. These results suggest that mis-targeting of RAG proteins to cRSSs within *TEL* and *AML1* translocation regions might be responsible for the t(12;21)(p13;q22) chromosomal translocation not bearing *Ig*/*TCR* regions.

Keywords: RAG; aberrant V(D)J recombination; cryptic RSS; chromosomal translocation

Introduction

V(D)J recombination is the mechanism for somatic generation of the vast antigen-receptor diversity during B- and T- lymphocyte development in vertebrate immune system[1]. V(D)J recombination can be divided into three steps, *i.e.*, DNA binding of RAG, RAG-mediated DNA cleavage, and ligation of two cleaved ends by the non-homologous end joining (NHEJ) machinery. At the first step, RAG1 and RAG2, lymphoid cell-specific recombinase proteins bind to recombination signal sequences (RSSs) present in recombination regions of *immunoglobulin* (*Ig*) or *T cell receptor* (*TCR*) genes. The RAG proteins then initiate recombination by introducing a nick precisely at the so-called heptamer sequence, and the generated 3' hydroxyl group attacks the opposite strand through transesterification, leading to double-strand break (DSB). At the final step, two RAG-cleaved DNA ends are joined by NHEJ. RSS is an essential part of V(D)J recombination and consists of conserved heptamer and nonamer elements separated by an intervening spacer of either 12±1 or 23±1 nucleotides, which are known as 12-RSS or 23-RSS, respectively [1; 2]. Although the AT-rich nonamer sequence (consensus sequence, 5'-ACAAAACC-3') is less conserved, the heptamer usually consists of the palindromic sequence (consensus sequence, 5'-CACAGTG-3'), with some sequence variation [3]. RAG1 recognizes and binds to both heptamer and nonamer sequences.

Intrinsically, V(D)J recombination precisely occurs only among V(D)J gene segments flanked by conserved RSSs. However, it is reported that V(D)J recombination is implicated in the formation of reciprocal chromosomal translocations by inappropriate targeting of V(D)J to non-antigen receptor loci [4], termed "aberrant or illegitimate V(D)J recombination". Indeed, recurrent chromosomal translocations between *Ig*/*TCR* loci and

proto-oncogenes are found to be hallmarks of lymphoid malignancies [5]. The presence of cryptic RSSs (cRSSs) close to the breakpoint sequences identified in some types of lymphoid malignancies suggests that RAG proteins bind to and cleave such cRSSs at the translocation region [6]. It is shown that RAG proteins specifically bind to and cleave the cRSSs identified near the translocation found in some lymphoid malignancies, and this cleavage occurs through the standard nick-hairpin mechanism *in vitro* [7]. However, a prediction rule in mis-targeting of RAG proteins to a RSS variant is presently unclear, since the knowledge on chromosomal translocation is mainly derived from post-translocation observation of breakpoints in leukemia patients. It should be examined in functional analyses whether cryptic RSSs are exactly involved in aberrant V(D)J recombination.

The t(12;21)(p13;q22), in which the *TEL* (*ETV6*) gene on chromosome 12 is rearranged with the *AML1* (*CBFA2*) gene on chromosome 21 resulting in *TEL-AML1* fusion gene, is the most frequent chromosomal translocation associated with pediatric B-cell precursor acute lymphoblastic leukemia [8]. The leukemogenic function of *TEL-AML1* has been well studied, but the timing and the precise molecular mechanism of the translocation including events from the cleavage to subsequent ligation generating *TEL-AML1* remain largely unknown. On this line, it is interesting to note that there are present several 5/7 matches for the consensus heptamer (5 nucleotides match out of 7 nucleotide-hepatmer consensus sequence) in the *TEL* and *AML1* breakpoint junctions [9]. Thus, we hypothesized that mis-targeting of RAG proteins to cRSSs contributes to the recombination in the translocation regions of *TEL* and *AML1*, and examined the hypothesis.

Materials and Methods

Plasmid construction

Single-strand cDNAs were amplified by PCR with specific primer sets: for full length RAG1 (1-1043 a.a), RAG1F125 and RAG1R primers were used;, for RAG1ΔN (380-1043 a.a), RAG1F1265 and RAG1R3269 primers, for full length RAG2 (1-527 a.a), RAG2F163 and RAG2R1779 primers, for RAG2ΔC (1-388 a.a), RAG2F163 and RAG2R1326 primers, respectively. RAG1ΔN and RAG2ΔC cDNAs were subcloned into pCHA vector [10]. cDNA for the catalytic mutant RAG1D711AΔN, containing a replaced amino acid mutation (D711A) in its $D^{603}D^{711}E^{965}$ motif was prepared by PCR with mutated oligonucleotides (RAG1D711A, 5'-GGC TAT GCT GAA AAA CTT GTG CG-3', where a mutated nucleotide is underlined), and then the PCR product was subcloned into pCHA vector. Sequences of all oligonucleotides used in this study are summarized in Supplementary Table 1.

Extrachromosomal recombination assay and analysis of recombinants

pAT plasmid vectors for the substrate of extrachromosomal V(D)J recombination assay were made by emulating pGG49 plasmid [11]. The detailed procedure for preparation and construction of pAT substrates is described in Supplementary Material and Methods.

293T cells were maintained in DMEM supplemented with 10% fetal calf serum and seeded into 6 cm-diameter dishes at 2.5 x 10^5 cells per dish. Cells were transfected with 5 μg of recombination substrates (pAT vectors) and 2.5 μg of each 'core' of RAG expression vectors (pCHA-RAG1ΔN and pCHA-RAG2ΔC) or "full-length" RAG expression vectors (pCHA-RAG1 and pCHA-RAG2) by the calcium phosphate method. Core RAG1 and RAG2

are sufficient to support the rearrangement in extrachromosomal V(D)J recombination assay [12]. After incubation at 37°C for 48 h, plasmid DNA was recovered from cells by rapid alkaline lysis and used for transformation. Bacterial transformants were plated on media containing ampicillin alone (100 μg/ml) and that containing ampicillin and tetracycllin (100 and 11 μg/ml, respectively). Recombined DNAs confer both ampicillin (A) and tetracycline resistance (T). The ratio of the number of A and T colonies (ATC) to A resistant colonies (AC) represents the recombination frequency.

Chromatin immunoprecipitation (ChIP) assay

293T cells were transfected with RAG expression vectors (pCHA-RAG1D711 and pCHA-RAG2) by Trans-IT293. Soluble chromatin was prepared from 293T cells ($4x10^6$) pre-fixed with 1% formaldehyde at room temperature for 10 min, and ChIP was carried out essentially according to the protocol from Upstate Biotechnology using rabbit anti-RAG1 antibody (described below). The recovered DNA was amplified by PCR with specific sets of primers for region A to H, TEL-F161 and TEL-R281, TEL-F2168 and TEL-R2803, TEL-F82 and TEL-R54, TEL-F3327 and TEL-R3448, TEL-F6690 and TEL-R6810, TEL-F9321 and TEL-R9447, TEL-F10573 and TEL-R10676, and TEL-F13040 and TEL-R13170, respectively. The PCR products were separated through 6% native-PAGE, visualized by staining with EtBr, and quantified with NIH Image. The purification and specificity of anti-RAG1 antibody is described in Supplementary Material and Methods.

Ligation-mediated (LM)-PCR

LM-PCR was performed essentially as previously described [13]. 293T cells were transfected with RAG expression vectors (pCHA-RAG1 and pCHA-RAG2) by Trans-IT293, and the genomic DNA was purified at 48 h post transfection. The purified genomic DNA (0.8 μg) was ligated to 20 pmol of linker DNA (BW linker), assembled from oligonucleotides BW-1 and BW-2 as described previously [13], by addition of T4 DNA ligase (5 units) (TOYOBO). After incubation at 16°C for 18 h, ligated products were subjected to PCR. Ligated DNA was amplified for 20 cycles with the linker specific primer (BW-1) and either one of distal *TEL* locus-specific primers, TEL-F161, TEL-F420, TEL-F9321, or TEL-F10573. Initial denaturation was performed at 94°C for 5 min, followed by 20 cycles of amplification (94°C for 30 s, 60°C for 30 s, and 72°C for 1 min). Nested PCR was also performed by 30 cycles of amplification (94°C for 30 s, 60°C for 30 s, and 72°C for 1 min), with the other linker specific primer (BW-1H) and either one of the proximal *TEL* locus-specific primers, TEL-F187, TEL-F3422, TEL-F9352, or TEL-F10593. PCR products were separated through 6% native polyacrylamide gels and visualized by staining with EtBr. As the positive control for DSB, we used *Dra*I (TOYOBO) for single DSB within the target loci. Serial dilution of *Dra*I-digested genomic *TEL* region was used for amount controls of quantitative determination of the amount of the RAG-dependent LM-PCR products.

Results

Extrachromosomal recombination assays for RAG-mediated translocation between TEL and AML1

To examine whether RAG proteins are involved in translocation between a pair of translocation regions, neither of which contains an antigen receptor locus as partner, we first evaluated the recombination frequency between a pair of ALL (acute lymphoid leukemia)- and AML (acute myeloid leukemia)-associated fusion genes quantitatively using an extrachromosomal recombination assay. We selected approximately 1 kbp-long DNA fragments which contain the regions dense in breakpoint junctions observed in *TEL-AML1* (t(12;21)(p13;q22)) and *E2A-PBX1* (t(1;19)(q23;p13)) from ALLs, and *SET-CAN* (t(9;9)(q34;q34)), *DEK-CAN* (t(6;9)(23;q24)), and *MLL-AF9* (t(9;11)(p22;q23)) from AMLs (Fig. 1 and Supplementary Fig. 1A). The schematic structure of plasmids is depicted in Supplementary Fig. 1B. Core RAG expression vectors along with a substrate were transfected to 293T cells. Core RAG1 and core RAG2, lacking the N-terminal region and the C-terminal region, respectively, have been proven to be the minimal regions sufficient for recombination of extrachromosomal substrates [14; 15]. In the case of pAT-*E2A-PBX1*, pAT-*SET-CAN*, pAT-*DEK-CAN*, and pAT-*MLL-AF9*, we did not detect any colony generated by recombination out of 10,000~100,000 ampicilline resistant colonies, indicating that the recombination frequency was below 0.001% (Table 1). In contrast, we detected small but distinct number of recombinants using pAT-*TEL-AML1*. We found that the recombination frequency between translocation regions of *TEL* and *AML1* is about 0.0083%, which is 50-fold lower than that of pAT-12-RSS-23-RSS and the recombination is dependent on expression of core RAG proteins

(Table 2). Several studies have shown that core RAG proteins increase the frequency of aberrant V(D)J recombination in endogenous receptor loci [16]. To confirm that RAG-dependent recombination of *TEL* and *AML1* is mediated evenly by core RAG and full-length RAG proteins, we carried out the extrachromosomal recombination assay with full-length RAG proteins using pAT-*TEL-AML1*. We detected full-length RAG-dependent recombination in pAT-*TEL-AML1* at almost the same frequency (0.0114%, data not shown) with that of core RAG proteins.

The core RAG-dependent *TEL-AML1* breakpoints were determined by DNA sequencing and mapped as shown in Fig. 1B. We found that the RAG-dependent breakpoints of *TEL* and *AML1* scatter on the cloned *TEL* and *AML1* DNA regions. Although there was a case in which the breakpoint is identical with that found in an ALL patient, the other identified breakpoints are not completely in agreement with *TEL-AML1* translocation breakpoints found in patients.

Several heptamer-like sequences are present in the vicinity of breakpoints within TEL and AML1

Next, we analyzed the nucleotide sequences of the junctions of *TEL* and *AML1* in six examples of RAG-dependently recombined DNA (Fig. 2). We sequenced all of the *TEL-AML1* breakpoints and we could not find any nucleotide addition or deletion. As shown previously in the breakpoints in *TEL-AML1* patients [9], we found heptamer-like sequences in the vicinity of RAG-dependent recombination regions as indicated in Fig. 2 (by *asterisks*). Any apparent nonamer or nonamer-like sequences were not found, and thus a heptamer-like

elements could be the target of RAG proteins. On the other hands, we could not find *Alu* repeats or Pu/Py tracts characteristic to *TEL-AML1* rearrangements. Thus, we speculated that the RAG-dependent recombination between *TEL* and *AMLl* loci may be mediated by mis-targeting of RAG proteins to these heptamer-like sequences.

RAG proteins bound to the TEL translocation region

In the course of RAG-mediated recombination, RAG proteins first bind to recombination sites. We next investigated whether RAG proteins are bound to nucleosomal DNA around *TEL* breakpoints in the nucleus. Since the region containing *AML1* breakpoints in *TEL-AML1* translocations is widely spread over 200 kbp-long intron 1, we focused on *TEL* breakpoints that are present densely in only 14 kbp-long intron 5. To examine the interaction of full-length RAG proteins with chromatin through the specific regions within the *TEL* intron5, we performed ChIP assays with rabbit anti-RAG1 antibody using 293T cells transiently expressing RAG1D711A and RAG2. RAG1D711A interacts with RAG2 and binds to DNA normally, but lacks the DNA cleavage activity *in vitro* (data not shown), so that we could detect RAG that is kept bound to DNA in ChIP assays. Specific primer sets for ChIP assays in *TEL* translocation regions are indicated in Fig. 3A by alphabets (A to H). Fig. 3B shows that RAG proteins are most at the region D, and relatively high at the region G, among regions so far tested. The region D is the most dense in breakpoints of *TEL-AML1* chromosomal translocation observed in ALL patients. These results indicate that RAG proteins selectively bind to the *TEL* hotspot region of *TEL-AML1* chromosomal translocation.

Recently, it has been shown that the C-terminal PHD finger of RAG2 recognizes

histone H3 trimetylated at lysine 4 (H3K4me3) [17], and it is conceivable that full-length RAG proteins used in our ChIP assays recognized H3K4me3 at the region D, but not DNA sequence. To examine this possibility, we performedwo ChIP assays with core RAG proteins and we found that core RAG proteins also most strongly bound to the region D (data not shown).

RAG proteins introduced DSBs to the TEL translocation region

Further to confirm the RAG-mediated recombination between *TEL* and *AML1* translocation regions, we examined the RAG-mediated DSBs on the *TEL* translocation region by LM-PCR assays. We selected four positions within *TEL* intron 5 as shown in Fig. 4A, and prepared *TEL* specific primers and a linker primer. 293T cells were transfected with full-length RAG expression vectors, and the genomic DNA was purified after 48 h post transfection. In the presence of RAG proteins, a strong band of LM-PCR products were detected in the region II (Fig. 4C, lane 7), which is the most dense in breakpoints of *TEL-AML1* among the four target positions. In contrast, any LM-PCR product was hardly seen in the other positions where no or fewer breakpoints were detected. *Dra*I restriction endonuclease was a positive control nuclease to generate a single band in this assay (Fig. 4C, lanes 3, 4, and 5), since each region has a *Dra*I site near the each primer (Fig. 4B). We calculated the frequency of RAG-mediated DSB at the highest site in region II by comparison with a dilution series of *Dra*I-digested genomic DNA, and we found that 3.4% of the genomic DNA was cleaved at the site dependently on the expression of RAG proteins. The sequencing analysis showed that these LM-PCR products were matched to the sequence within region II (data not shown). Therefore, we conclude that the expression of RAG proteins specifically introduces

DSBs within the nucleosomal translocation region II of *TEL*, which is dense in breakpoints observed in *TEL-AML1*-positive ALL patients. Our findings suggest that RAG proteins introduce DSBs into the chromatin DNA of *TEL* translocation regions, where *TEL-AML1* breakpoints are localized densely.

Discussion

A number of translocations associated with lymphoid malignancies have been proposed to be due to targeting mistakes of RAG proteins. For example, an RSS of the *Ig*/*TCR* gene segment and an RSS-like motif near the proto-oncogene sequence are eventually used. For characterization of such RAG-dependent recombination, the extrachromosomal recombination assay has been proven to be useful [6; 18; 19]. However, there have been few report that the aberrant recombination activity of RAG could contribute to the chromosomal translocation not bearing *Ig*/*TCR* loci. Thus, we applied this assay system to ALL and AML patient-associated breakpoint regions where no consensus RSS is present. We have demonstrated that the recombination through *TEL* and *AML1* breakpoints occurs by expression of RAG proteins. As we used non-lymphoid cells for the extrachromosomal recombination assay, it also eliminates the possibility that other lymphoid cell-specific factors are necessary for the recombination between *TEL* and *AML1*. It is documented that t(12;21)(p13;q22) translocation is frequently observed in ALL [20], and RAG proteins are expressed in *TEL-AML1*-positive Reh cells. Therefore, our results might explain this B-cell specific appearance. Furthermore, we have shown that RAG proteins introduce DSBs into the *TEL* translocation region dense in breakpoints of *TEL-AML1* chromosomal translocation. Taken together, it is possible that aberrant RAG-mediated DNA cleavage is a direct cause of the chromosomal translocation between *TEL* and *AML1* gene loci.

Nadel and Lewis have categorized RAG-mediated aberrant V(D)J recombination into two classes, “Type 1” and “Type 2” [18; 21; 22]. Type1 translocation is caused by targeting mistake of the RAG proteins on one proto-oncogene and one *Ig/TCR* gene segment,

while type 2 translocation could be due to a repair mistake of the V(D)J recombination between a set of proto-oncogene and *Ig/TCR* gene segment and an additional *Ig/TCR* fragment. The *TEL* and *AML1* translocation at hotspots on the plasmid vector undergo in the RAG-dependent manner without any *Ig*/*TCR* gene segments, suggesting the *TEL-AML1* recombination was generated mainly by mis-targeting of RAG proteins (type 1), rather than the illegitimate repair of the V(D)J intermediates (type 2). However, we cannot exclude the contribution of the type 2 mechanism in *TEL-AML1* translocation, since we did not use the *Ig*/*TCR* gene segments as a substrate for the type 2 recombination in our recombination assay. In addition to the classical NHEJ pathway after RAG-shepherded DSBs, alternative DNA joining pathways such as homologous recombination and alternative NHEJ are known to be subjected to unstable RAG-mediated nicking [23]. Recently, it has been shown that a non-consensus heptamer element destabilizes a post-cleavage complex and allows coding and signal ends to be joined by alternative DNA repair pathways [24]. We could find only a limited terminal microhomology in the junction of *TEL-AML1* recombination, so that RAG-dependent *TEL-AML1* recombination might be ended with alternative NHEJ.

In summary, findings in this study support the possibility that mis-targeting of RAG proteins to the heptamer-like sequences within *TEL* and *AML1* translocation regions could contribute to the *TEL-AML1* chromosomal translocation.

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Figure legends

Fig. 1. Schematic maps of *TEL* and *AML1* loci used in this study.

(**A**) Breakpoints of *TEL-AML1* translocation in patients. The loci within *TEL* and *AML1* translocation regions chosen for the extrachromosomal recombination assay are indicated by black bars, respectively. The major breakpoints (arrowheads) in *TEL* and *AML1* are located in intron 5 and intron 1, respectively. The distribution of the breakpoints along *TEL* intron 5 and *AML1* intron1 from individual patients containing *TEL-AML1* translocation is taken from previous reports. The exons are shown as solid boxes.

(**B**) Nucleotide sequences around breakpoints within *TEL* and *AML1* translocation regions used for the extrachromosomal recombination assay. Open arrowheads indicate breakpoints found in patients, and closed arrowheads show the RAG-dependent recombination sites identified in the extrachromosomal recombination assay. Nucleotide sequences of the top strands of the *TEL* and *AML1* are indicated.

Fig. 2. Fused sequences of *TEL* and *AML1* by RAG-dependent recombination.

Six sequence examples flanking the breakpoints of RAG-dependent *TEL-AML1* are shown. Nucleotide homologies in the genomic sequence are represented by vertical bars. Nucleotide sequences with at least 5/7 match to the consensus heptamer element (CAC A/T GTG) are indicated by asterisks.

Fig. 3. RAG proteins bind to *TEL* translocation region.

(**A**) The positions of locus-specific primers in the *TEL* intron 5 for the ChIP assays (A to H).

(**B**) The relative amounts of RAG-bound DNA on the *TEL*. Association of RAG with the *TEL* translocation region in 293T cells was examined by ChIP assays. ChIP assays were performed using either rabbit pre-immune serum or rabbit anti-RAG1 antibody. 293T cells were transfected with expression vectors encoding RAG proteins (HA-RAG1D711A and HA-RAG2) with a mutation destroying the cleavage activity. The genomic DNA was purified at 48 h post transfection. Immunoprecipitated DNA was amplified with PCR using sets of primers specific for the regions from A to H in panel A. PCR products were analyzed by a 6% native-PAGE, visualized by staining with EtBr, and quantified with NIH Image. The amount of DNA immunoprecipitated with rabbit anti-RAG1 antibody was represented as a ratio to that with control rabbit pre-immune serum. Experiments were repeated three times.

Fig. 4. Double-stand breaks within the genomic *TEL* locus dense in translocation region by RAG proteins.

(**A**) The position of locus-specific primers in the *TEL* intron 5 for LM-PCR (black bars, I-IV) are shown.

(**B**) The 200 bp-long DNA sequences of target regions of LM-PCR. The DNA sequences of 1st and 2nd specific primers are indicated by italic letters and underlined, respectively. A *Dra*I site involved in each region is indicated by bold letters.

(**C**) RAG protein-mediated DSBs in the *TEL* translocation region. 293T cells were transfected with the expression vectors encoding RAG proteins (HA-RAG1 and HA-RAG2), and genomic DNA was purified at 48 h post transfection. The purified DNA was ligated with linker DNA, and then amplified by PCR using a linker specific primer and a *TEL* locus specific primer.

PCR products were separated through 6% native polyacrylamide gels with 50 bp ladder marker. Digestion of genomic DNA by *Dra*I provides a fragment near one of primers. Each position of *Dra*I-digested LM-PCR product is indicated by arrowhead. The most distinct LM-PCR product in region II is indicated by asterisk, and was quantitatively analyzed by Image Gauge analysis software (Fujifilm, Tokyo, Japan). By comparing the amount of the PCR product with that derived from a dilution series of *Dra*I-digested products, the ratio of DSB was calculated, and is indicated in the bottom of the region II panel. This figure is a typical one among three to five experiments.

Table legends

Table 1. The recombination frequencies of genes associated with some translocations in the extachromosomal recombination assay.

Non-lymphoid 293T cells derived from human embryonic kidney cells were transfected with a pAT plasmid substrate with or without expression vectors of core RAG. After 48 h of transfection, plasmid minichromosomes were harvested by the rapid alkaline lysis method, and used for transformation of *E.coli* in the presence of ampicillin or both ampicillin and tetracycline on LB agar plates. The recombination frequency (ATC/AC x 100) was calculated as the ratio of the number of both ampicillin- and tetracycline-resistant colonies (designated ATC) relative to that of the ampicillin-resistant colonies (designated AC). A total of >100,000 ampicilin resistant colonies were counted for each of four independent experiments. More than three independent assays were performed for each set of translocation genes. The indicated frequencies represent the result of one typical experiment. "12-RSSmut" is a mutant sequence of 12-RSS, in which the first two nucleotides of heptamer (5'-CA-3') was replaced with the 5'-AC-3' nucleotides.

Table 2. The recombination frequency of pAT-*TEL-AML1* substrate.

The extrachromosomal recombination assay was carried out using pAT-*TEL-AML1* plasmid and core RAG protein expression vectors. R'; average recombination frequency (%). The R' value of pAT-12-RSS-23-RSS in the presence of RAG was 0.45.

Table 1.

pAT vector	AC	ATC	(ATC/AC)x100
12-RSS-23-RSS	110800	498	0.44946
12-RSSmut-23-RSS	90800	0	<0.00110
E2A-PBX1	11200	0	<0.00090
SET-CAN	95200	0	<0.00105
DEK-CAN	59200	0	<0.00169
MLL-AF9	133300	0	<0.00075

Table 2.

	AC	ATC	(ATC/AC)x100	R'
RAG -	76600	0	<0.0013	
	46700	0	<0.0021	<0.0024
	26190	0	<0.0038	
	46400	0	<0.0022	
RAG+	88400	8	0.0090	
	72180	7	0.0098	
	71550	5	0.0070	0.0083
	39780	3	0.0075	