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RESEARCH ARTICLE

Viral sequence integration into introns of chemokine receptor genes

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Abstract

Viral DNA sequences are able to integrate into the non-coding DNA sections of the genome of human cells which have been infected, either spontaneously or experimentally. We have made a data-base search for integration events of non-endogenous viruses into the introns of chemokine receptor sequences. A BLAST search of all viral DNA sequences, using the intronic sequences as "Query," returned several significant alignments. However, due to the high reiteration rate of the non-coding sequences in the human genome, it became necessary to re-examine the individual alignments to verify whether the virus-flanking intronic sequence was really located in a chemokine receptor intron. We found only one unquestionable event of viral insertion of a section of a long terminal repeat of the murine leukemia virus within the first intron of the CC chemokine receptor 7 gene. Possible biological effects of such an insertion are discussed. Further experimental or clinical research could demonstrate the occurrence of other intronic viral insertions in human chemokine receptor genes.

Keywords: *Viral insertion sites; chemokine receptor genes; introns; human*

Abbreviations: *AIDS acquired immunodeficiency syndrome; bp base pairs; CCR5 CC chemokine receptor 5; CCR7 CC chemokine receptor 7; CCR10, CC chemokine receptor 10; CXCR5 CXC chemokine receptor 5; HERV human endogenous retrovirus; HIV-1 human immunodeficiency virus type-1; MLV, murine leukemia virus; nt nucleotide(s)*

Introduction

RNA-transcribed DNA sequences of retroviruses and DNA sequences of dsDNA viruses are able to integrate into the introns of genes of human infected cells. This finding has attracted considerable interest because

(a) it explains why some viral infections may remain latent for long periods of time,[1] and

(b) viral integration may provide a means to introduce nucleotide (nt) sequences of therapeutic value, linked to appropriately engineered "non-harming" viruses (vectors), into the genome of human cells carrying adverse mutations in their DNA (gene therapy).[2–5]

Gene therapy by means of viral carriers has proven successful in some pathological conditions (e.g., children with X-linked inherited severe combined immunodeficiency (X-SCID) could be cured by gene transfer into primitive hemopoietic cells) and presently different hematological disorders and cancers seem to be amenable to gene therapy approaches.[5–8]

However, the process of integration may bring about alterations in the genomes of the host cell and the integrating virus itself. For instance, in one reported case Epstein-Barr virus integration caused the deletion of more than 15 kb of cellular DNA[9] and in another case human papillomavirus type 18 integration was accompanied by loss of part of the sequence coding for the E1 viral protein.[10]

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In addition, intronic viral integration may affect the processes of translation of the exons of the gene involved and/or the neighboring genes. Avian leukosis virus (a retrovirus) insertion in the fourth intron of the tyrosinase gene, by causing tyrosinase gene aberrant transcripts lacking exon 5, is responsible for the recessive white mutation in chickens. A closer analysis revealed that none of the five exon sequences was altered, but the viral insertion blocked the expression of exon 5 apparently by activating one of the three alternate polyadenylation sites that are present in the normal intron 4 sequence.[11, 12]

Tsukahara et al. reported that murine leukemia virus (MLV, a retrovirus) integration in introns may dysregulate the mRNA-levels of T-cell clones.[13] Human immunodeficiency virus type-1 (HIV-1, a retrovirus) insertions within host introns may trigger mechanisms contributing, via the dysregulation of the expression of the genes involved or of other neighboring genes, to the pathogenesis certain acquired immunodeficiency syndrome (AIDS)-related diseases.[14]

In a clinical trial, aimed at curing patients with a severe combined immunodeficiency (SCID)-X1 disease by means of the introduction of IL-2 receptor gamma cDNA incorporated in a gammaretroviral vector derived from the Moloney MLV, in some patients the vector integrated near the LIM domain–only 2 (*LMO2*) or the *BMI1* or the *CCND2* proto-oncogenes and brought about dysregulation of several proteins, including cytoplasmic membrane, transcriptional control, cell cycle control and tumor suppressor proteins. As a result, these patients developed a T cell leukemia within some months after the treatment.[15, 16] For further discussion of the genotoxicity of viral integrations into introns, consult. [2, 17–25]

The site of gene integration of viral vectors is not strictly determined but it is not completely random, since each vector may exhibit preferred sites of integration.[20] Furthermore, in some cases, the gene with dysregulated expression is quite distant (e.g., 100,000 base pairs (100 Kbp) from the integration site), while other intervening genes are not apparently altered. [2]

Although acute viral integration (either spontaneous or experimental) may or may not lead to a dysregulation of both host and viral biological functions, in the long terms of mammals and viruses co-evolution other biological equilibriums have been achieved. Some viruses predated from eukaryotic cells sequences coding for chemokine receptor-like proteins, this "molecular piracy" being probably occurring in order to partly escape the immune surveillance.[26–35]

On the other hand, some infecting viruses became permanently integrated into the genome of humans (and other mammals) as "human endogenous retroviruses" (HERVs), but these were domesticated and rendered non-pathogenic by some modification in their genes. For instance, in the human HERV HML6 a few frame shifts (due to insertions) and nonsense mutations disrupted the reading frame of the essential gag, pro and pol genes.[36] It is even suspected that these insertions (which are very likely footprints of ancient germ-cell infections by active retroviruses ("fossil viruses") and may account for 1% to 5% of the human genome) may have been converted into positive players in the regulation of some normal biological processes of eukaryotic cells, such as differentiation and regulation of gene expression, through their promoter, enhancer, hormone responsive element and polyadenylation signal sequences. However, although most HERVs are defective due to the accumulation of mutations, deletions, and termination signals, a limited number of HERVs (e.g., the HERV-K superfamily) have the potential to synthesize viral products and have been implicated in certain cancers and autoimmune diseases.[37–41]

Here we report on a systematic data-base search for viral integrations within the introns of the chemokine receptor genes. Human endogenous viruses are not considered in this study and will be dealt with in a separate paper. Chemokine receptors are involved in innate immune responses as well as in the adaptive immunity.[42, 43] Although the ligand specificity is not strict and several chemokines may ligate different receptors of the chemokine receptor subfamily,[44] a dysregulation of only a single receptor may affect the immune response.[32, 45, 46]

Paradoxically, at least in one case the block of a chemokine receptor might even be beneficial: the CC chemokine receptor 5 (CCR5) serves as co-receptor for the cellular entry of the HIV-1 and subjects lacking functional CCR5 show decreased susceptibility to HIV-1 infection while not exhibiting any apparent immune defect.[31] Accordingly, a number of therapeutic strategies have been developed to block CCR5 function and delay AIDS progression.[47].

Strategy for the search for intronic virus integration sites in chemokine receptor genes

All DNA nt sequences were derived from NCBI GenBank (http://www.ncbi.nlm.nih.gov) as on October 31, 2008. NCBI BLAST tools (Human Genome and Nucleotide-nucleotide BLAST [blastn]) were used.

The following human chemokine receptors exhibit one or two introns: CC receptor 2 (CCR2, Chromosome: 3, Location: 3p21.31), CC receptor 6 (CCR6, Chromosome: 6, Location: 6q27), CC receptor 7 (CCR7, Chromosome: 17, Location: 17q12-q21), CC receptor 9 (CCR9, Chromosome: 3, Location: 3p21.3), CC receptor 10 (CCR10, Chromosome: 17, Location: 17q21.1-q21.3), CXC receptor 4 (CXCR4, Chromosome: 2, Location:

2q21), CXC receptor 5 (CXCR5, Chromosome: 11, Location: 11q23.3). All other human chemokine receptors are intronless.

The first step of the search was aimed at spotting the position of each intron of interest in the relevant chromosome using as tool BLAST Assembled Genomes - Human. For instance, it was determined that the first intron of human CCR7 exactly matches NT_010755.15 Homo sapiens chromosome 17, from 2,439,491 to 2,445,947. The second intron of human CCR7 exactly matches NT_010755.15 Homo sapiens chromosome 17, from 2,436,367 to 2,439,440.

The second step was a BLAST (blastn) search for significant alignments between each intron (the "Query") and the data-base of viral sequences (Taxid: 10239). This step returns significant alignments between the bases of the human intron under study and the bases of any data-base sequence immediately adjacent to a viral sequence. Non-human sequences and human endogenous virus sequences were discarded and a check was made to remove any viral "contamination." CCR2, CCR6, and CXCR4 introns did not yield significant alignments.

All other intron sequences exhibited several significant partial alignments with human/viral (insertional) sequences. For example, (among other results) nt 2640-2731 of the first intron of CCR7 align with nt 44-135 of AB257911 Homo sapiens, murine leukemia virus integration site (Identities = 100%, Gaps = 0%, Expect = 2e-38) (First sequence) and nt 2778-2982 of the second intron align with nt 828-1034 of AF339139 Homo sapiens, Human papillomavirus type 18 integration site 4 (Identities = 73%, Gaps = 3%, Expect = 8e-21) (Second sequence).

Even high percentages of identity or very low E-values do not guarantee per se that the insertion is actually within the tested intron. Indeed, sequences in the non-coding sections of the genome are often highly reiterated with only a few nt differences. For instance, one of the intron-insertional (for simian immunodeficiency virus) sequence alignments we found in this screen gave 100 % of Query coverage and Identity in the human chromosomes 2, 3, 7, 18, 19, with E-values ranging from 9e-49 to 1e-53, furthermore, it gave a 100 % of Query coverage with Identities >95 % and E-values ranging from 3e-49 to 9e-55 in human chromosomes 1, 4, 5, 10, 12, 16, 17, being also significantly represented in most of the other chromosomes. The assumption that an E-value of 10^{-25} or lower may be sufficient to define unequivocally the insertion site [10] is not warranted.

Thus, in the third step of the search the "Sbjct" sequences returned by the previous step (which are identical or significantly similar to sections of the intron under consideration) were used as Queries to "fish" for the more likely human chromosome(s) where such sequences could be located (a blastn search). The very best-aligning chromosomes returned (Sbjct) were considered. If none of them corresponded to the location of the chemokine receptor gene under consideration, the search was stopped at this stage. On the contrary, if a significant alignment was found within the chromosome normally carrying the chemokine receptor gene under consideration, the search was continued by carrying out a fourth step. For example, both sequences First and Second (derived in step 2) align preferentially with human chromosome 17 where the CCR7 gene is located.

In the fourth step the "Sbjct" sequences obtained from the previous step were tested in order to spot their positions in the chromosome involved, using a combination of the Human Genome BLAST tool and the PubMed-GENE data-base. When the sequence was unequivocally spotted within the intron of the chemokine receptor under investigation, the search was ended and the virus integration within this intron was regarded as demonstrated. In the cases of location at other sites within the same chromosome, the distance (in bp) from the insertion site and the intron under consideration was calculated. For instance, the First sequence of the example aligns (100%) with NT_010755.15 Homo sapiens chromosome 17, from 2,443,217 to 2,443,308 and thus falls in the first intron of CCR7 (see the first step). On the contrary, the Second sequence of the example aligns (100%) with NT_010755.15 Homo sapiens chromosome 17, from 1,570,651 to 1,570,845 and thus falls at a distance of about 869 Kbp from the CCR7 gene.

Results

Due to the high redundancy of non-coding sequences, only a fraction of the several "significant" (in terms of length and E-value) alignments between the individual chemokine receptor introns and the human sequences containing viral insertions could be (putatively) attributed to the chromosome containing the chemokine receptor gene under investigation. Of the remaining sequences only one insertion was unquestionably located in the context of a chemokine receptor intron. The sequence AB257911.1 (Homo sapiens DNA, MLV integration site, partial sequence, clone: MLV-TPA-Mat-035) was derived from a human T-cell line experimentally infected with the MLV.[13] The whole sequence is comprised of 135 bp. The first 43 nt correspond exactly (100 %) to a MLV (a retro-transcribing virus) long terminal repeat (LTR). The remaining 92 nt correspond exactly (100 %) to nt 2640-2731 of the first intron of the human CCR7 gene (EF064758.1), while no other human sequence aligns for more than 25 % with this sequence. The total length of the first intron is 6457 bp and thus the virus is integrated at about one third of the length of the intron.

The sequence AY255031.1 (Homo sapiens clone AD-13 HIV-1 integration site) was mapped to human chromosome 17q21.2,[14] a location which might correspond to the CCR10 or CCR7 genes. However, the best alignment (93 % over 267 nt) was with an intronic section of the Homo sapiens enhancer of the zeste homolog 1 (Drosophila) (EZH1) gene (NM_001991.3). This gene lies at a distance of approximately 50 Kbp from the CCR10 gene, which might thus be indirectly affected.

Other viral insertions in the chromosomes containing the chemokine receptor genes under consideration were too distant from the latter to be taken into further consideration.

Discussion

The present data-base search for viral integrations into the introns of human chemokine receptor genes (excluding human endogenous viruses) returned only a single unquestionable example of integration of a MLV vector into the first intron of CCR7. Of course, it is possible that further experimental or clinical research will demonstrate other examples of viral integration into chemokine receptor gene introns. Furthermore, the data-base search is liable to be affected by some false-negative results: in fact, the viral insertion may have somewhat distorted the intron sequence and, due to the vast partial redundancy of non-coding sequences in the human genome, even small alterations may render the real integration site unrecognizable.

The instance of viral integration described in the Results section deserves further analysis, for three main reasons:

(a) MLV-based vectors are often considered as tools for gene therapy delivery,

(b) possible alterations in CCR7 expression as a consequence of the viral integration may bring about severe immune disorders, and

(c) expression alterations may also affect immediately neighboring genes, some of which are involved in DNA transcription or tumor progression.

CCR7 is a member of the chemokine receptor subfamily, which in turn belongs to the very large family of the seven-transmembrane (serpentine), G-protein coupled receptors (GPCR) of class A (rhodopsin-like).[31] CCR7 is expressed on all naive T cells, some memory T cells, B cells, and mature dendritic cells. The typical CCR7 ligands are chemokine ligands 19 and 21 (CCL19/ELC and CCL21/6Ckine). CCL19 is expressed predominately by stromal cells within the T-cell zones of lymph nodes, spleen, and Peyer's patches, CCL21 is expressed in the high endothelial venules of lymph nodes and Peyer's patches, in the T-cell zones of spleen, lymph nodes, and Peyer's patches and in the lymphatic endothelium of multiple organs. On the whole, the most abundant expression of both chemokines is in lymph nodes.[48, 49]

Functionally, CCR7 and its ligands play a pivotal role in the recruitment of naive T cells and antigen-activated dendritic cells to the T cell-rich areas of secondary lymphoid organs. Due to an impaired migration of lymphocytes, CCR7-deficient mice reveal profound morphological alterations in all secondary lymphoid organs and show severely delayed antibody response kinetics.[50]

In addition, due to impaired chemotactic migration, lymphocytes accumulate in peripheral tissues, such as the stomach (where they may form massive lymphoid aggregates, reminiscent of a hypertrophic gastropathy,[51] or the lungs.[52] Interestingly, during embryogenesis, mesenchymal cells at the prospective sites of lymphoid organ development express ligands for both CXCR5 and CCR7[53], further investigation demonstrated that although the CXCR5 system is possibly the major player, the CCR7 system likely exerts a significant cooperative function in lymphoid organ organogenesis and organization.[54] In particular, CCR7 regulates the functional organization of the thymus architecture and CCR7-deficient mice suffer generalized multi-organ autoimmunity pathologies.[55, 56] Distinct yet cooperative roles of CXCR5 and CCR7 have also been demonstrated in the early immunity response to bacterial pathogens.[57]

Although most reports emphasize the role of CCR7 in eliciting appropriate immune responses, other recent studies indicate that, at least in some cases, adaptive immunity may develop through alternative pathways which do not involve CCR7. Peripheral antigens can diffuse in soluble form into the secondary lymphoid organs where, after acquisition, processing, and presentation by the resident antigen-presenting cells, they can induce immune responses. Furthermore, alternative chemoattractant receptors may substitute for CCR7 in inducing the homing of T cell and antigen-presenting cells into the secondary lymphoid organs.[58]

CCR7 may also be expressed in the cells of some primary tumors, such as gastric, rectal, breast and skin tumors. Under these conditions lymph node metastases are more frequent, possibly due to a chemotactic attraction of tumor cells by the corresponding CCLs produced by the lymphatic endothelium and the lymph nodes.[48,59–65] Use of a siRNA to block CCR7 expression at the mRNA level impaired colon cancer invasion and inhibited lymph node metastases of colon cancer cells.[66] Thus, the CCR7 protein has been proposed as a possible target for anticancer mAb therapy.[67]

Conclusion

CCR7 plays a significant homing role in lymphoid organ organogenesis and in triggering immune responses, but its expression by cancer cells may facilitate their metastatic spread to lymph nodes. Natural mutations of the CCR7 gene have been described, e.g., a truncation in a human colorectal cancer which makes the receptor unable to localize to the cell surface and renders it non-reactive to external ligands.[68]

Viral insertions in the CCR7 introns might in principle affect normal gene transcription or translation, thus in some way altering the immune responses. Furthermore, viral insertions in the CCR7 introns might lead to over- or under-expression of some important neighboring genes, including the retinoic acid receptor alpha gene (which is involved in the control of the basic transcription machinery), the topoisomerase (DNA) II alpha gene (which controls and alters the topologic states of DNA during transcription), and the tensin 4 gene (whose expression is correlated with tumor progression). In humans all these genes are located at chromosome 17q21 and 230 Kbp or less away from the CCR7 gene (map of chromosome 17: http://www.ncbi.nlm.nih.gov, Gene).

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