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Mechanisms Leading to Nonrandom, Nonhomologous Chromosomal Translocations in Leukemia

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Abstract

Nonrandom, reciprocal translocations between nonhomologous chromosomes are critical cellular events that lead to malignant transformation. Therefore, understanding the mechanisms involved in these chromosomal rearrangements is essential for understanding the process of carcinogenesis. There has been substantial discussion in the literature over the past ten years about mechanisms involved in constitutional chromosomal rearrangements, including deletions, duplications, and translocations. Yet our understanding of the mechanisms of chromosomal rearrangements in cancer is still developing. This review presents what is known about the mechanisms involved in selected nonrandom chromosomal translocations in leukemia.

Keywords

cytogenetics; translocation; DNA repair; leukemia; Alu

1. Brief Introduction to Mechanisms Leading to Nonrandom Chromosomal Rearrangements in Cancer

Reciprocal translocations between nonhomologous chromosomes have been observed in a variety of malignancies, including leukemia, lymphoma, and sarcomas and they have been implicated in the etiology of disease. Over the past four decades, it has become clear that acquired, nonrandom chromosome abnormalities are associated with specific cancers, including hematologic malignancies, as can be gleaned from the literature annotated at the outstanding internet site, <http://cgap.nci.nih.gov/Chromosomes/Mitelman> [1] and summarized at <http://atlasgeneticsoncology.org/> [2]. Nonrandom chromosomal translocations associated with cancer are critical cellular events that lead to malignant transformation. Therefore, understanding the mechanisms involved in nonrandom chromosomal translocations between nonhomologous chromosomes is essential for understanding the process of carcinogenesis. There has been substantial discussion in the literature over the past ten years about mechanisms involved in constitutional chromosomal rearrangements, including deletions, duplications, and translocations (reviewed by [3]). Yet, our understanding of the mechanisms of chromosomal rearrangements in cancer remains incomplete. This review attempts to present what is known

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about the mechanisms involved in nonrandom chromosomal translocations in selected types of leukemia.

2. Introduction to Reciprocal Translocations between Nonhomologous Chromosomes in Hematologic Malignancies. The Philadelphia (Chromosome) Story

Cancer is a genetic disease of somatic cells. Chromosomal abnormalities in cancer cells are usually acquired in somatic cells rather than constitutional in origin. Chromosomal alterations and karyotypic instability in human tumor cells have been investigated for more than a century. In 1890, David Hanseemann reported that tumor cells with an abnormal chromosome constitution (aneuploidy) had abnormal mitotic spindles with extra poles and other chromosome segregational abnormalities [4]. This aneuploidy theory was formalized in the early 1900s when Theodor Boveri, while studying chromosomal segregation in *Ascaris* worms and *Paracentrotus* sea urchins, suggested that malignant tumors arise from a single cell that has acquired an abnormal chromosome constitution [5]. Chromosomal cytology was moved forward in the 1920s and 1930s by two technical improvements by plant cytologists, 1) the squash technique which eliminated the difficulty in reconstructing and analyzing chromosomes from serially-sectioned preparations and 2) the application of the mitotic inhibitor, colchicine, derived from the Mediterranean *Colchicum* plant, to disrupt the mitotic spindle and increase the yield of mitotic cells available for chromosome analysis [6]. In spite of these improvements, mammalian cytogenetics did not blossom until the 1950s, when hypotonic treatment was found to aid in chromosome spreading, enabling visualization and analysis of individual chromosomes. In 1955, Jo Hin Tjio and Albert Levan applied these new advances in cytogenetics to their cultures of human fetal lung tissues and discovered that the human chromosome number is 46, rather than the previously reported 48 [7]. In 1960, Peter Nowell and David Hungerford reported the first consistent chromosome abnormality in malignant human cells (chronic granulocytic leukemia), later called the Philadelphia (Ph) chromosome in chronic myelogenous leukemia (CML) [8]. Their finding supported Boveri's hypothesis that cancer cells express chromosomal alterations. Major advances in cancer cytogenetics occurred in the 1970s as a result of the development of chromosomal banding methods which enabled the identification of individual chromosomes as unique entities based on their staining patterns. Banding also enabled Janet Rowley to define the Ph translocation as a reciprocal translocation between chromosomes 9 and 22, t(9;22)(q34;q11) [9], although the breakpoints of this translocation have been revised to t(9;22)(q34;q11.2) [10]. The Ph chromosome is the small der(22)t(9;22)(q34;q11.2). Identification of the Ph translocation, whether by classical or molecular cytogenetic methods or molecular genetic techniques, is now essential for diagnosis of patients with CML [11]. Clarification of the cytogenetic aberrations in CML led to molecular characterization of the breakpoints, identification of the genes involved, and description of the molecular pathology of the malignancy. In CML, cloning of the Philadelphia translocation breakpoint revealed that the translocation creates a hybrid gene consisting of 5' regulatory and coding sequences of the *BCR* gene on chromosome 22 joined with 3' coding, polyadenylation, and termination sequences from the *ABL1* proto-oncogene on chromosome 9 (reviewed in [12–14]). The t(9;22)(q34;q11.2) translocation is observed in more than 90% of CML patients, 25% of acute lymphocytic leukemia (ALL) patients including 20% of adult ALL patients, and 5% of childhood ALL patients, and more rarely in patients with chronic neutrophilic leukemia and topoisomerase II (topo II) inhibitor therapy-related leukemia [14,15]. Nora Heisterkamp and John Groffen determined that the Philadelphia translocation involves the *ABL1* gene and is a reciprocal translocation between chromosomes 9 and 22, with the small der(22) producing the key transcript that leads to cellular transformation (reviewed in [13]). They also identified and named the *BCR* gene. Other investigators determined that the *BCR/ABL1* gene produces a hybrid mRNA molecule and that the resulting protein is a tyrosine kinase that leads to

transformation. The chimeric gene is comprised of a constant *ABL1* segment from exons 2 to 11 and a variable *BCR* segment. There are two breakpoint cluster regions in the *BCR* gene, with the major breakpoint cluster region joining all of *BCR* up to exons 13 or 14 to *ABL1* from exon 2 to 11, resulting in the p210^{BCR-ABL}. The minor breakpoint cluster in *BCR* joins the first exon of *BCR* to *ABL1* from exons 2–11, resulting in the p190^{BCR-ABL}. Breaks in micro-*BCR* join all of *BCR* up to exon 19 to *ABL1* (exons 2–11), resulting in the p230^{BCR-ABL} (reviewed in [14]). The p210^{BCR-ABL} and p190^{BCR-ABL} proteins are constitutively active protein kinases, with the p190^{BCR-ABL} protein having higher activity, resulting in an aggressive acute leukemia phenotype compared to the p210^{Bcr-Abl} protein, which results in chronic leukemia. Heisterkamp and Groffen and another group were the first to determine that the BCR-ABL protein produced by the hybrid gene caused leukemia [12]. Although this constitutionally activated tyrosine kinase signals multiple cellular pathways, its transforming activities are dependent solely on its tyrosine kinase activity.

CML was the first disease for which targeted molecular therapy was designed (reviewed in [14,16]). As reviewed by Brian Druker [16], the BCR-ABL tyrosine kinase was found to be selectively inhibited by STI571 (now Gleevec®, Glivec®, or imatinib mesylate) from Ciba-Geigy (now Novartis). Druker identified STI571, the precursor to imatinib, as a promising anticancer compound for its ability to kill CML cells by turning off the signal of the BCR-ABL tyrosine kinase. Preclinical studies showed that imatinib specifically inhibits the proliferation of cells expressing the BCR-ABL kinase *in vitro* and the growth of *BCR/ABL1*-caused tumors *in vivo* while minimally inhibiting the colony forming potential of normal bone marrow. Imatinib inhibits the BCR-ABL tyrosine kinase, blocks cellular proliferation, and induces apoptosis in cells expressing the Ph chromosome. Imatinib is the first approved drug to directly turn off the signal of a protein known to cause a cancer. Druker also conducted the first clinical trials of imatinib. The Phase I trials of imatinib showed an amazing response, with 98% of patients treated daily with ≥300 mg achieving a complete hematologic response, with 96% of those responses lasting more than one year, and 55% of myeloid blast crisis patients responding to the same dose, with 18% having responses that lasted more than one year. The Phase II studies confirmed the results of the Phase I studies and served as the basis for FDA approval of the drug in 2001. Although a minority of patients have experienced insensitivity or resistance to imatinib due to mutations in sites in the kinase that result in failure to bind to imatinib, amplification of the kinase at the genomic level, or overexpression of the transcript could also lead to this outcome. Druker [16] emphasizes that the vast majority of CML patients in the chronic phase achieve a complete cytogenetic response from imatinib and few relapse.

Thus, clarification of the cytogenetic aberrations in a cancer enables not only diagnosis, but molecular characterization of the breakpoints, identification of the genes involved, description of the molecular pathology of the malignancy, and development of targeted therapies. But, by what mechanism do these nonrandom cytogenetic rearrangements arise?

3. From Chromosome Breakage to Mechanisms of Nonrandom Chromosomal Rearrangement

For a translocation to be recognized clinically, two or more chromosomes must 1) undergo ‘contemporaneous’ chromosomal breakage and ligation to each other and 2) confer a proliferative or survival advantage, enabling a cell to undergo clonal expansion, resulting in a clinical phenotype, and enabling detection by a cytogenetics laboratory. Chromosomal translocations are thought to arise by a DNA double strand break (DSB) followed by aberrant DNA repair. However, for nonhomologous chromosomes to join, they must break at about the same time while either located near each other in the interphase nucleus or be brought together by some mechanism. As reviewed by Aplan [17], recent studies have shown that the *BCR* and *ABL1* loci are localized close to each other in CD34+ bone marrow cells (see also Meaburn et

al., this issue). Similarly, *MYC* and *BCL6*, two genes that are frequently rearranged with the *IGH* locus in B-cells, are all also located in proximity to each other. An alternative mechanism was shown by Aten *et al.* [18] during an investigation of changes in DSB track morphology after treatment with alpha-irradiation. They demonstrated that DSB-containing chromosome domains are mobile. They also showed that DSB-containing chromosome domains marked with antibodies to γ -H2AX (a modified histone protein that localizes to the sites of DSB) cluster, which they suggested occurs by an adhesion process involving the MRE11 complex [18]. These results support a hypothesis that suggests that the two nonhomologous chromosomes each undergo a DSB first and then are clustered by an unknown mechanism and fused by 'misrepair' [18].

Understanding this 'misrepair' of chromosomal breaks necessitates a thorough knowledge of the DNA damage response. For many years, cytogeneticists have known that patients with 'chromosome breakage' syndromes express chromosomal translocations and have a higher risk of leukemia. For example, Fanconi anemia patients are prone to developing myelodysplastic anemia (MDS) and acute myeloid leukemia (AML), which resemble those that are secondary to therapy with alkylating agents (t-MDS, t-AML) [19,20]. Bloom syndrome patients develop both myeloid and lymphoid leukemias, with the cytogenetic alterations in AML resembling those in t-AML [21]. Patients with ataxia telangiectasia (AT), with mutations in their *ATM* genes, develop primarily B-cell lymphomas and T-cell leukemias of the T-CLL type [22]. The increase in frequency of lymphoid malignancies in AT patients appears to be a consequence of abnormal rejoining of genes during V(D)J recombination of immunoglobulin or T-cell receptor (TCR) genes, including TCR α (TRA@) at 14q11.2, TCR β (TRB@) at 7q34, and TCR γ (TRG@) at 7p14, although the most frequent breakpoint in the abnormal rejoining involves the *TCL1A* gene at 14q32.1. Many sporadic cases of leukemia, including those in individuals later found to be *ATM* mutation carriers, express loss of one copy of the *ATM* gene. Loss or mutation of *ATM* results in a poor prognosis (shorter survival and more aggressive disease) in B-cell chronic lymphocytic leukemia primarily as a result of resistance to DNA damage-based chemotherapy [23]. Although the mechanism is not clear from the literature, our own unpublished results in head and neck cancer give us a clue that this may result from downregulation of the ATM pathway and upregulation of the ATR pathway, leading to chemo- and radioresistance as a result of 'misrepair' and continued proliferation of the increasingly more aberrant cells. Thus, it appears that the DNA damage response, including the ATM and ATR signaling pathways, is critical in the development and response to therapy of hematologic malignancies.

Only in the past decade have the features of the 'chromosome breakage' syndromes been utilized to define defects in the DNA damage response in cancer cells. Causes of DNA damage include attack by ultraviolet light, ionizing radiation, topoisomerase II inhibitors or environmental mutagens and cellular errors, such as base pair mismatch during DNA replication, errors during V(D)J recombination, replication fork collapse, or defects caused by naturally occurring reactive oxygen species. Common chromosomal motifs at breakpoint regions may lead to double strand breaks and nonhomologous chromosomal translocations [15]. These include topo II and DNase I cleavage sites and scaffold-associated regions (SARs), which are reported to closely associate with the *BCR* and *ABL1* breakpoints. Topo II and scaffold protein II are essential for chromosome condensation. Aberrant activity of these elements may also result in DSB, as a result of torsional stress in DNA not relieved by topo II, for example [24]. The DSB usually leads to a cascade of cellular events (the DNA damage response) that results in repair of the damage or cell death. Failure in the DNA damage response and DSB repair can lead to genetic alteration or chromosomal instability, including nonhomologous chromosomal translocations, resulting in neoplastic transformation.

The DNA damage response involves the sensing of DNA damage followed by transduction of the damage signal to a network of cellular pathways, from those involved in the cellular survival response, including cell cycle checkpoints, DNA repair, and stress responses to telomere maintenance, and the apoptotic pathway. At the apex of the DNA damage response are the ataxia telangiectasia mutated (*ATM*) and ataxia telangiectasia and Rad3-related (*ATR*) genes. *ATM* and *ATR* are phosphatidylinositol 3-kinases and phosphorylate their substrates in response to DNA damage. *ATM* is a critical regulator of cellular responses to DSB-induced DNA damage by agents such as ionizing radiation (IR) [25,26]. Following activation by IR, *ATM* kinase initiates a cascade of signaling pathways that culminates in cell cycle arrest, apoptosis and DNA repair. *ATM* triggers these responses by phosphorylating its substrates, the ABL, p53, p95/NBS/nigrin, BRCA1, and CHK2 proteins (and others) [27,28]. Defects in the cellular response to DSB result in genetic mutations, gene amplification, and chromosomal aberrations, which in turn lead to malignant transformation and tumorigenesis [26]. *ATM* has more than 30 known substrates and others are being discovered at a rapid rate (reviewed in [25]). The *ATR* gene codes for the *ATR* protein which directs a pathway that responds primarily to ultraviolet radiation and agents that block replication fork progression, such as aphidicolin and hydroxyurea. The *ATR* pathway appears increasingly to be intertwined with the *ATM* pathway [25]. *ATR* has been shown to be a critical factor in the maintenance of chromosomal integrity. Inhibition of *ATR* function leads to chromosomal instability, overexpression of common fragile sites [29], which in turn is thought to result in chromosomal rearrangement, including gene amplification in cancer cells [30].

Mammalian cells use two pathways to repair DSB, the high-fidelity homologous recombination repair (HRR) pathway that acts during the late S and G2 phases of the cell cycle and the nonhomologous end joining (NHEJ) pathway, which is an error-prone pathway that functions throughout the cell cycle [31]. In HRR, a homologous DNA strand is used as a template for repair of double strand breaks and is therefore, essentially error-free [21]. NHEJ is the prevalent DSB repair process in mammalian cells and is also essential for the repair of DSB during V(D)J recombination [21]. In NHEJ, end joining reactions between nonhomologous chromosomes result in translocations that are characterized by deletions of up to 100 bp and also, regions of DNA sequence microhomology of up to 7 bp in length [32,33]. Patients with Fanconi anemia and Bloom syndrome have defects in the HRR pathway and appear to upregulate their NHEJ pathway. The relationship between the error-prone NHEJ pathway and chromosomal instability in these two syndromes is clear, but the exact relationship between the increased frequency of 'misrepair' in patients with these two disorders and the specific chromosomal alterations seen in their leukemias (-7,7q- and others) is not entirely clear.

There is evidence that breakpoint translocation junctions of a number of leukemias are characterized by regions of DNA sequence microhomology, a feature typical of NHEJ. These include the *BCR/ABL1* rearrangement in CML, the *TEL/AML1* breakpoint in acute lymphocytic leukemia (ALL), and the *PML/RARA* translocation in acute promyelocytic leukemia (APL) [15,21]. Although it appears that these translocations between nonhomologous chromosomes are mediated by NHEJ, exactly how the breakpoints are brought together is not entirely clear, although there has been substantial literature on the subject. As discussed at the beginning of this section, breakpoints can either be located near each other in the interphase nucleus or be brought together by an active process. Careful sequencing of the DNA at breakpoints in CML shows that *Alu* sequences are located near the chromosomal breakpoints in *BCR* and *ABL1* and keep these chromosomal segments near each other in the nucleus, possibly facilitating recombination [32,34]. In addition, five *BCR/ABL1* rearrangements reported by Jeffs *et al.* [35] and Martinelli *et al.* [34] carried translin (TSN) protein or translin-related protein (TSNAX) binding sequences on both sides of the rearranged genes, suggesting that these sequences may be important in CML. The *BCR* and *ABL1* genes are being transcribed and thus,

having an open structure, may be prone to recombination as a result of their proximity due to the *Alu* sequences. They proposed that a looped-out chromatin conformation during interphase, possibly as a result of the *Alu* repeat distribution, may expose the breakpoint regions and enable sequence-specific, possibly *Alu*-mediated mechanisms of DNA recombination to occur [35]. Jeffs *et al.* indicated that the results of this and their earlier studies suggest that *BCR/ABL1* recombination may be facilitated by specific motifs, such as the translin-related protein binding sequences in the recombination regions, close homology to which is also seen in the nearby *Alu* sequences. Similarly, Martinelli *et al.* [34] suggested that the presence of binding sites for recombinatory proteins, such as translin, in the vicinity of the *Alu* sequences could mediate recombination at the site of chromosomal breaks in the open structure genes, resulting in reciprocal translocations. Translin is an octomeric protein that binds to sequences at chromosomal breakpoint junctions, forming higher oligomeric complexes and clamping together free chromosomal ends, facilitating repair [36]. The protein has a preference for repeat sequences, including chromosomal breakpoint consensus sequences, novel conserved sequence motifs, and simple GC or AT repeats. Sequencing of breakpoints in hematologic malignancies, including CML and AML and in solid tumors, such as liposarcomas and rhabdomyosarcoma has revealed extensive homology with translin binding consensus sequences, suggesting that translin mediates nonhomologous chromosomal translocations in these malignancies (reviewed in [36]). The binding sequences of translin are short and common in the human genome, estimated to occur every 57.1 bases. The translin protein binds to simple sequence repeat motifs even more avidly than to the consensus sequence and to both blunt-ended and tailed DNA duplexes [36]. Therefore, the combination of open chromatin, *Alu* sequences, and translin binding consensus sequences may result in coupling of the ‘contemporaneous’ broken ends of nonhomologous chromosomes, resulting in a translocation. Strick *et al.* [15] explained that scaffold-associated regions (SARs) are AT-rich segments of DNA located at the bases of interphase and metaphase chromatin loops. They are regions of open chromatin as defined by DNase I hypersensitive sites and the sites of topoisomerase II (topo II) chromatin cleavage sites. SARs have been proposed to be sites of chromosomal fragility. These authors described that *ABL1* has SARs associated with breakpoint cluster regions, although *BCR* does not. Both *BCR* and *ABL1* are characterized by open and accessible chromatin regions as defined by topo II drug-induced DNA cleavage and DNase I sensitivity. There is evidence that *Alu* sequences can function like SARs, supporting the idea that alignment could occur between an *ABL1* SAR and an *Alu* sequence near the BCR major breakpoint cluster region [15]. Jasin and colleagues [37] developed an intron-based system to induce chromosomal translocations at *Alu* elements and found that when DSB were introduced adjacent to identical *Alu* elements, translocations occurred at high frequency primarily from repair by the single strand annealing (SSA) pathway (85%). When the *Alu* elements were divergent, the translocation frequency was the same, but the repair and translocation occurred primarily by the NHEJ pathway (93%). The SSA pathway can also occur at homologous sequences near a DSB, but unlike HRR which involves strand invasion, it involves annealing of DNA strands formed after resection at the DSB. Their results suggest the possibility that apparent inter-*Alu* recombination events between heterologous elements may arise from microhomology-mediated NHEJ in which the two *Alu* elements are in register with each other [37].

Thus, some nonhomologous chromosomal translocations appear to be mediated by coupling of repeat sequences, either inter-*Alu* or *Alu*-SAR, that could bring the breakpoints into proximity. This inter-*Alu* mechanism has been reported to be involved in the only known recurrent constitutional non-Robertsonian translocation in humans, the t(11;22)(q23;q11.2) [38]. Slightly different from the observation in malignancies, the breakpoints are in the *Alu* motif itself. Both *Alu* repeats involved in the constitutional t(11;22) contain an *Alu* ‘core’ sequence, comprised of the pentanucleotide motif, CCAGC. In fact, a recent report by Kurahashi *et al.* [39] showed that this translocation is mediated by palindromes. The breakpoint

region in chromosomal band 11q23 constitutes a nearly perfect palindrome and thus, has been designated as a palindromic AT-rich repeat on 11q23 (PATRR11). A similar palindromic region was also found surrounding the breakpoint on 22q11.2 (PATRR22), although it had no homology with PATRR11. The cloned breakpoint sequences adopt a cruciform configuration *in vitro*. Since the DSB appear to occur at the center of both palindromic regions, followed by repair via the NHEJ pathway, these authors propose that the symmetric PATRR is likely to adopt a cruciform structure in male meiotic cells, resulting in genetic instability that leads to the recurrent t(11;22) [39].

The relatively prevalent chromosomal translocations that lead to therapy-related leukemia after topo II inhibitor therapy are thought to result from the treatment ([40] and many others). Translocations involving the *MLL* gene are amongst the most frequent nonrandom translocations in hematologic malignancies [40]. *MLL* rearrangements are seen in a wide array of malignancies, including 3–10% of AML, T-cell ALL, 8–10% of B-cell ALL, MDS, lymphoblastic lymphoma, and Burkitt lymphoma. *MLL* is quite ‘promiscuous,’ in that it rearranges with more than 60 partner genes. Leukemias with *MLL* rearrangements are unusual because of their biphenotypic immunophenotype, and have unique clinical presentation in infants where they are seen in about 80% of acute leukemias, and in about 25% of therapy related leukemias, especially after treatment with topo II inhibitors. Rearrangements involving *MLL* have come in many forms, including translocations, insertions, duplications, partial tandem duplications, amplifications, and ‘jumping translocations’ comprised of an integration of an amplicon of amplified *MLL* and neighboring genes inserted into one or more chromosomes [40]. Topo II normally functions as a homodimeric enzyme that catalyzes the relaxation of supercoiled DNA by a three-step reaction, comprised of double strand cleavage (with a 4 bp staggered nick), strand passage, and strand re-ligation. This would result in perfect or near-perfect interchromosomal exchange. Such events have been identified in the *NUP98* and *MLL* loci in patients with t-AML after therapy with topo II inhibitors. Although many of the *MLL* rearrangement breakpoints map to an 8.3 kb breakpoint cluster region, most t(4;11) (q21;q23) *MLL* rearrangements either in infants or adults are not perfect, containing short deletions, inversions, insertions, direct repeats, and microhomology at the breakpoints, suggestive of single or double strand breaks followed by NHEJ [41]. Further speculation concerns the observation that the cleavage site in *MLL* maps to the base of a SAR, is in an open configuration, and therefore, more susceptible to cleavage by various enzymes, including apoptotic nucleases. The SARs are thought to contain regions that become single stranded under stress [41]. These results may implicate a role for SSA in *MLL* rearrangements, similar to the inter-*Alu* recombination discussed above. Chromosomal translocation mediated through interchromosomal recombination between *Alu* elements in somatic cells is reported to be relatively rare [41]. It has been implicated in complex *BCR/ABL1* rearrangements and in one case of AML with an *MLL/AF9* translocation. In contrast, the partial tandem duplication (PTD) of *MLL* is usually mediated by inter-or intrachromosomal recombination between *Alu* motifs within the *MLL* gene, consistent with SSA in seven of nine *MLL* PTD patients and NHEJ between *Alu* and non-*Alu* sequences in two other cases [41]. Whether by NHEJ or SSA, the translocation junctions between nonhomologous chromosomes contain errors. If those translocations endow a cell with a proliferative advantage resulting in a clonal outgrowth detectable as a result of clinical phenotype, we call this cancer. Further studies of the mechanisms of chromosomal rearrangement and defects in the DNA damage response in cancer cells are warranted to deepen our understanding of neoplasia and enhance our ability to develop tools for early diagnosis, prognostic markers, and targeted therapies.

6. Conclusion

Translocations between nonhomologous chromosomes are etiologic in many hematologic malignancies. The mechanism(s) by which these chromosomal translocations form is/are not

entirely clear, but we do know that they arise from coupling of contemporaneous broken chromosome ends located near each other in the interphase nucleus, characterized by the combination of open chromatin, *Alu* or other repeat sequences, and translin binding consensus sequences. Further studies are warranted to better understand the chromosomal domain locations in interphase nuclei from different cell types, the means by which broken chromosome ends may be brought together in an active process, the details of interactions between SARs, *Alu* and other repeat sequences, and translin and other binding sites and their roles in NHEJ and/or SSA. Only then can the mechanisms leading to nonrandom nonhomologous chromosomal translocations in leukemia be understood.

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References

1. Mitelman, F.; Johansson, B.; Mertens, F. Mitelman Database of Chromosome Aberrations in Cancer. 2006. <http://cgap.nci.nih.gov/Chromosomes/Mitelman>
2. Huret, J-L. Atlas of Genetics and Cytogenetics in Oncology and Haematology. 2006. <http://atlasgeneticsoncology.org/>
3. Lupski JR, Stankiewicz P. Genomic disorders: Molecular mechanisms for rearrangements and conveyed phenotypes. PLoS Genet 2005;1(6):e49. [PubMed: 16444292]
4. Hanseemann D. Ueber asymmetrische celltheilung in epithel krebsen und deren biologische bedeutung. Virchows Archiv für Pathologische Anatomie 1890;119:299–326.
5. Boveri, T. The Origin of Malignant Tumors, by Theodor Boveri... translated by Marcella Boveri. Baltimore: The Williams & Wilkins Company; 1929.
6. Hsu, TC. Human and Mammalian Cytogenetics. New York: Springer-Verlag; 1979.
7. Tjio JH, Levan A. The chromosome number of man. Hereditas 1956;42:1–6.
8. Nowell PC, Hungerford DA. A minute chromosome in human chronic granulocytic leukemia. Science 1960;132:1497.
9. Rowley JD. A new consistent chromosomal abnormality in chronic myelogenous leukemia identified by quinacrine fluorescence and Giemsa staining. Nature 1973;243:290–3. [PubMed: 4126434]
10. Shaffer, LG.; Tommerup, N., editors. ISCN. An International System for Human Cytogenetic Nomenclature. Basel: S. Karger; 2005. 2005.
11. Jaffe, ES.; Harris, NL.; Stein, H.; Vardiman, JW., editors. WHO Classification of Tumors. Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues. Lyon: IARC Press; 2001.
12. Heisterkamp N, Groffen J. Molecular insights into the Philadelphia translocation. Hematologic Pathology 1991;5:1–10. [PubMed: 2050600]
13. Heisterkamp N, Groffen J. Philadelphia-positive leukemia: a personal perspective. Oncogene 2002;21:8536–40. [PubMed: 12476299]
14. Kurzock R, Kantarjian HM, Druker BJ, Talpaz M. Philadelphia chromosome-positive leukemias: From basic mechanisms to molecular therapeutics. Ann Intern Med 2003;138:819–30. [PubMed: 12755554]
15. Strick R, Zhang Y, Emmanuel N, Strissel PL. Common chromatin structures at breakpoint cluster regions may lead to chromosomal translocations found in chronic and acute leukemias. Hum Genet 2006;119:479–95. [PubMed: 16572268]
16. Druker BJ. Inhibition of the Bcr-Abl tyrosine kinase as a therapeutic strategy for CML. Oncogene 2002;21:8541–6. [PubMed: 12476300]
17. Aplan PD. Causes of oncogenic chromosomal translocation. Trends in Genet 2006;22:46–55. [PubMed: 16257470]

18. Aten JA, Stap J, Krawczyk PM, van Oven CH, Hoebe RA, Essers J, Kanaar R. Dynamics of DNA double strand breaks revealed by clustering of damaged chromosome domains. *Science* 2004;303:92–5. [PubMed: 14704429]
19. Tischkowitz M, Dokal I. Fanconi anaemia and leukaemia – clinical and molecular aspects. *Brit J Haematol* 2004;126:176–91. [PubMed: 15238138]
20. Bagby GC, Alter BP. Fanconi anemia. *Semin Hematol* 2006;43:147–156. [PubMed: 16822457]
21. Rassool FV. DNA double strand breaks (DSB) and non-homologous end joining (NHEJ) pathways in human leukemia. *Cancer Letters* 2003;193:1–9. [PubMed: 12691817]
22. Taylor AM, Metcalfe JA, Thick J, Mak YF. Leukemia and lymphoma in ataxia telangiectasia. *Blood* 1996;87:423–38. [PubMed: 8555463]
23. Bouley J, Deriano L, Delic J, Merle-Beral H. New molecular markers in resistant B-CLL. *Leuk Lymphoma* 2006;47:791–801. [PubMed: 16753863]
24. Penserga ETP, Skorski T. Fusion tyrosine kinases: a result and cause of genomic instability. *Oncogene*. 2006In Press.
25. Shiloh Y. The ATM-mediated DNA-damage response: taking shape. *Trends Biochem Sci* 2006;31:402–10. [PubMed: 16774833]
26. Zhou BB, Elledge SJ. The DNA damage response: putting checkpoints in perspective. *Nature* 2000;408:433–9. [PubMed: 11100718]
27. Khanna KK, Jackson SP. DNA double-strand breaks: signaling, repair and the cancer connection. *Nat Genet* 2001;27:247–54. [PubMed: 11242102]
28. Baskaran R, Wood LD, Whitaker LL, et al. Ataxia telangiectasia mutant protein activates c-Abl tyrosine kinase in response to ionizing radiation. *Nature* 1997;387:516–9. [PubMed: 9168116]
29. Glover TW, Arlt MF, Casper AM, Durkin SG. Mechanisms of common fragile site instability. *Hum Mol Genet* 2005;14:R197–205. [PubMed: 16244318]
30. Coquelle A, Pipiras E, Toledo F, et al. Expression of fragile sites triggers intrachromosomal mammalian gene amplification and sets boundaries to early amplicons. *Cell* 1997;89:215–25. [PubMed: 9108477]
31. Bernstein C, Bernstein H, Payne CM, Garewal H. DNA repair/pro-apoptotic dual-role proteins in five major DNA repair pathways: fail safe protection against carcinogenesis. *Mut Res* 2002;511:145–78. [PubMed: 12052432]
32. Weinstock DM, Richardson CA, Elliott B, Jasin M. Modeling oncogenic translocations: Distinct roles for double-strand break repair pathways in translocation formation in mammalian cells. *DNA Repair*. 2006In press
33. Weinstock DM, Elliott B, Jasin M. A model of oncogenic rearrangements: differences between chromosomal translocation mechanisms and simple double-strand break repair. *Blood* 2006;107:777–80. [PubMed: 16195334]
34. Martinelli G, Terragna C, Amabile M, Montefusco V, Testoni N, Ottaviani E, De Vivo A, Mianulli A, Saglio G, Tura S. Alu and translin recognition site sequences flanking translocation sites in a novel type of chimeric bcr-abl transcript suggest a possible general mechanism for bcr-abl breakpoints. *Haematologica* 2000;85:40–6. [PubMed: 10629590]
35. Jeffs AR, Wells E, Morris CM. Nonrandom distribution of interspersed repeat elements in the BCR and ABL1 genes and its relation to breakpoint cluster regions. *Genes Chromosomes Cancer* 2001;32:144–54. [PubMed: 11550282]
36. Sengupta K, Rao J. Translin binding to DNA: Recruitment through DNA ends and consequent conformational transitions. *Biochem* 2002;41:15315–26. [PubMed: 12484770]
37. Elliott B, Richardson C, Jasin M. Chromosomal translocation mechanisms at intronic Alu elements in mammalian cells. *Molec Cell* 2005;17:885–94. [PubMed: 15780943]
38. Hill AS, Foot NJ, Chaplin TL, Young BD. The most frequent constitutional translocation in humans, the t(11;22)(q23;q11) is due to a highly specific Alu-mediated recombination. *Hum Molec Genet* 2000;9:1525–32. [PubMed: 10888603]
39. Kurahashi H, Inagaki H, Ohye T, Kogo H, Kato T, Emanuel BS. Chromosomal translocations mediated by palindromic DNA. *Cell Cycle* 2006;5:1297–303. [PubMed: 16760666]

40. Aplan PD. Chromosomal translocations involving the MLL gene: Molecular mechanisms. DNA Repair. 2006In Press
41. Strout MP, Marcucci G, Bloomfield CD, Caligiuri MA. The partial tandem duplication of ALL1 (MLL) is consistently generated by Alu-mediated homologous recombination in acute myeloid leukemia. Proc Natl Acad Sci USA 1998;95:2390–5. [PubMed: 9482895]