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REVIEW

Mechanisms and impacts of chromosomal translocations in cancers

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Abstract **Chromosomal aberrations have been associated with cancer development since their discovery more than a hundred years ago. Chromosomal translocations, a type of particular structural changes involving heterologous chromosomes, have made a critical impact on diagnosis, prognosis and treatment of cancers. For example, the discovery of translocation between chromosomes 9 and 22 and the subsequent success of targeting the fusion product BCR-ABL transformed the therapy for chronic myelogenous leukemia. In the past few decades, tremendous progress has been achieved towards elucidating the mechanism causing chromosomal translocations. This review focuses on the basic mechanisms underlying the generation of chromosomal translocations. In particular, the contribution of frequency of DNA double strand breaks and spatial proximity of translocating loci is discussed.**

Keywords DNA double strand breaks; chromosomal translocations; genomic instability; spatial proximity; carcinogenesis

Introduction

Chromosomal translocation is a type of genetic and structural alteration occurring between heterologous chromosomes, the presence of which has been closely associated with carcinogenesis, especially leukemogenesis and lymphomagenesis. Chromosomal translocation often endows cancer cells with new biologic features, which is of important significance in cancer diagnosis and therapy. However, the molecular mechanisms causing chromosomal translocations have not been completely understood. The formation of chromosomal translocation involves a complicated process, initiated by induction of DNA double strand breaks (DSBs) and completed via re-joining of broken DNA ends on heterologous chromosomes. This review provides a historic perspective of discovering chromosomal translocation and covers recent progresses with a focus on the molecular mechanisms that induce the formation of chromosomal translocation. In the end, the important applications of chromosomal translocation in clinical medicine are also reviewed.

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Early work of genomic instability: the cause or the consequence?

Chromosomal abnormalities were initially observed as novel chromosomes in tumor cells in the late nineteenth and early twentieth centuries [1,2]. In 1890, the German biologist David von Hansemann found that tumor cells with chromosome abnormalities often contained several spindle bodies and other mitotic aberrations [2]. Then, the German cytologist Theodor Boveri, who is considered by many to be first cancer geneticist, provided the most comprehensive analysis of the data with most complete descriptions of tumor cell chromosomes [1,2]. Furthermore, Boveri proposed that tumor cells possessed "growth-stimulatory chromosomes" that were the cause of malignant transformation [1]. However, at that time, no specific markers were available to identify individual human chromosomes, so Boveri was not able to characterize the chromosomal changes in tumors in more depth.

Little was made of Boveri's observation until the 1950s, when several scientists discovered that virtually all tumor cell lines had chromosomal aberrations, frequently containing more than 100 chromosomes per cell, including dicentric and ring chromosomes [3]. However, cell lines from the same tumor type did not have the same aberrations, so these abnormal karyotypes were assumed to be a result of the inherent genomic instability of cancer cells, rather than a cause of malignant transformation. This view persisted until

the early 1970s, then, the chromosome banding techniques were invented, which allowed the identification of individual chromosomes and regions of chromosomes involved in aberrations [2]. In addition, these techniques enabled the researchers to associate specific chromosome abnormalities with human leukemias, lymphomas and solid tumors [2]. Subsequent studies convincingly showed that chromosomal translocations, a type of structural changes involving heterologous chromosomes, play a critical role in initiating cancers (reviewed in Ref. 4). Recently, with the application of next generation sequencing technique, the cancer genomes are being revealed in more depth at an almost revolutionary speed [5,6], which allows the identification of genomic aberrations at nucleotide level. These sequencing data suggest that cancer genomes are extremely complicated and composed of a large number of genetic changes [7–10]. Somatic mutations are common in cancers including point mutations, insertions, deletions, amplifications, translocations and copy number changes [7–10]. Given the complexity of cancer genomes, the role of genomic instability in cancer development needs more investigations.

Discovery of chromosomal translocation

In 1960, Peter Nowell and David Hungerford first reported that a minute chromosome was consistently associated with a human malignant disease, chronic myelogenous leukemia (CML) [11,12]. This peculiar chromosome in CML patients became known as the Philadelphia (Ph) chromosome [12]. Initially, in more than 10 years, it was thought that this small chromosome was caused by a simple deletion, and the loss of DNA from the chromosome was proposed to be the cause of the leukemia [2]. However, the application of chromosome banding techniques showed that the Ph chromosome was not caused by a deletion, instead, by an interchange between the long arms of chromosome (Chr) 9 and Chr 22 [13], thus, resulting in a reciprocal chromosomal translocation. This CML-associated t(9;22) translocation was discovered by Rowley in 1973 [13]. Around the similar time, the first translocation t(8;21) was also reported by Rowley, which was associated with acute myelogenous leukemia (AML) [14]. Recently, recurrent chromosomal translocations were also identified in solid tumors such as prostate cancers [15] and lung cancers [16,17].

Subsequent studies identified the genes involved in t(9;22) translocation by cloning the breakpoints, one of which was ABL1 gene (V-abl Abelson murine leukemia viral oncogene homolog 1) located on Chr 9, encoding the human cellular homolog of the transforming sequence of Abelson murine leukemia virus (A-MuLV), while another was the "breakpoint cluster region" (BCR) gene located on Chr 22 [18,19]. The reciprocal translocation between Chr 9 and 22 fuses the ABL1 gene from Chr 9 into the BCR gene on Chr 22, which creates a fusion transcript composed of the 5′ part of BCR and the 3′ part of ABL1 [20]. The common breakpoints in the BCR gene are located downstream of exons e13 or e14 (M-BCR) and less frequent breakpoints are downstream of exons e1 and e2 (m-BCR). Breakpoints in the ABL1 gene occur commonly upstream of exon a2, or of exon a3 in less than 5% of CML patients [21]. The ABL1 gene encodes a protein tyrosine kinase [22,23]. The BCR-ABL1 fusion gene still retains kinase activity [19,24]. However, the 5′ portion of ABL1 containing a SH3 domain is frequently deleted in the translocation. Activity of ABL1 protein is negatively regulated by its SH3 domain, and deletion of the SH3 domain causes ABL1 to become an oncogene [25–29]. Later studies showed that the BCR-ABL fusion caused by t(9;22) was essential for the development of CML [30]. Apart from associating with CML, the t(9;22) translocation was also found in acute lymphoblastic leukemia (ALL), especially in adult patients [31]. The identification of the cancer-causing fusion protein as a tyrosine kinase that is specifically expressed by cancer cells made it an attractive therapeutic target [30]. The development of the kinase inhibitor STI-571 has transformed CML therapy, which induces remission in majority of patients [30]. In addition, STI-571 might also be useful in treating ALL when combined with other drugs [32]. More importantly, the scientific success of the Philadelphia story proved the principle of targeted cancer therapy by discovering the cancer-causing mechanisms and searching for specific therapeutic approaches, and once more validated the power of basic research. Although it took several decades for this story to unfold, the paradigm it established may be followed for many other recurrent chromosomal structural changes associated with human cancers (see below).

Although the t(8;21) and t(9;22) were among the first translocations to be discovered, the first molecularly characterized translocation was the t(8;14) translocation associated with Burkitt's lymphoma (BL) [33,34], a type of mature B cell lymphoma. The genes involved in this translocation are the well-known oncogene *c-myc* located on Chr 8 and immunoglobulin heavy chain (*Igh*) gene on Chr 14 [33]. In 1982, Dalla-Favera and Croce showed that the human *c-myc* oncogene was located on the region of Chr 8 affected by the translocation, which was the first oncogene cloned at a translocation site [33]. At the same time, Phil Leder's group [35] showed that *c-myc* was translocated into the 5′ region of the *Igh* gene. This *Igh-c-myc* translocation established a paradigm for subsequent chromosomal translocations observed in many different types of mature B cell lymphomas [36]. Unlike the t(9;22) translocation, the *Igh-c-myc* translocation does not create a fusion protein, instead, it juxtaposes the *c-myc* oncogene next to the enhancer region of *Igh* locus, resulting in abnormal levels of *c-myc* oncogene expression [37]. Later studies show that the *Igh-c-myc* translocation is also observed in other types of mature B cell lymphomas such as diffuse large B cell lymphomas (DLBCLs) [38] and constitutes one of the most important components of malignant transformation [39,40]. We recently

established a mouse model based on the conditional deletion of *Xrcc4*, a DNA repair factor, in p53 deficient B cells, which recurrently developed peripheral B cell lymphomas termed CXP lymphomas [41]. Interestingly, these CXP lymphomas frequently harbor the reciprocal *Igh-c-myc* translocations and activate the expression of *c-myc* oncogene in a similar manner as in human mature B cell lymphomas [36,41,42]. The expression of c-myc oncogene can be activated by intronic Eμ enhancer (iEu) and *Igh* 3′ regulatory region (*Igh3′RR*) since transgenic mice that harbor c-myc oncogene fused with iEμ or Igh3′RR sequences are susceptible to B cell lymphomas [39,40,43–45]. Recent studies introduced an *Igh3′RR* inactivating mutation, which deletes the key hs3b and hs4 enhancers via gene targeting, into the CXP model to test its essential role in B cell lymphomagenesis [46]. These studies demonstrated that the *Igh3′RR* is not required for the generation of CSR-related *Igh* DSBs or *Igh-c-myc* translocations, but required for the activation and selection of *Igh-c-myc* translocations in CXP lymphomas [46].

Cloning of translocation breakpoints has discovered new oncogenes that are involved in regulating cell growth and inducing malignant transformation. For example, the AML-associated t(8;21) translocation led to the identification of AML1, an important transcription factor in hematopoietic lineage [47]. In fact, leukemia and lymphoma are now the most extensively characterized human malignant diseases [2], chromosomal translocations that alter either the function or expression of involved genes play a critical role in the etiology of these diseases [36]. However, the mechanisms causing chromosomal translocations are still not completely understood. Studies show that antigen receptor loci are frequently involved in chromosomal translocations such as t(14;18) translocation occurring between *Igh* and *bcl-2*, the well-known anti-apoptotic gene, which is associated with follicular lymphomas [48,49]. Evidence suggests that translocations at antigen receptor loci in lymphoid cells are likely caused by mistakes during lymphocyte specific DNA recombination processes [50,51] (see below). However, the precise mechanisms that underlie most recurrent translocations including both driver and passenger translocations have not yet been elucidated [51].

Mechanisms for translocations involving antigen receptor loci

The underlying molecular mechanisms for translocations frequently occurring at antigen receptor loci in lymphocytes have been extensively reviewed [50–54]. Thus, only a few basic principles of lymphocyte-specific recombination processes are discussed here. T and B lymphocytes are the major components of our immune system, which can mount highly specific immune responses against invading pathogens by generating a nearly infinite diversity of antigen receptors within the limits of a finite genome. This amazing diversity of adaptive immunity is largely achieved through lymphocyte specific DNA recombination process at antigen receptor loci, known as V(D)J recombination [55]. V(D)J recombination occurs in both T and B cell progenitors and assembles the variable region exon from V, D and J gene segments that are responsible for antigen recognition [55] (Fig. 1). In antibody-producing B cells, assembly of the variable region exons of *IgH* and immunoglobulin light (*IgL*) chain genes occurs in B lymphocyte progenitors during early development in bone marrow (BM) [56,57]. Productive $V_H(D)J_H$ rearrangements in pro-B cells signal differentiation to the pre-B cell stage where *IgL* genes are assembled [58]. There are two *IgL* loci, *Igκ* and *Igλ*. In mice and humans, *Igκ* genes are generally rearranged before *Igλ* genes [56].

In periphery, upon antigen activation, mature B cells undergo *IgH* class switch recombination (CSR), another DNA recombination process that further diversifies the constant region of *IgH* gene (C_H) [59]. Both V(D)J recombination and CSR involve a cut-and-join mechanism and generate DNA DSBs as an intermediate [50]. Classical non-homologous end joining (C-NHEJ) repairs the DSBs introduced, respectively, by the recombination activating genes (RAGs) in V, D, J gene segments during V(D)J recombination or activation induced deaminase (AID) in large repetitive switch regions (S) that lie upstream of each of the various sets of C_H exons during CSR (Fig. 1). XRCC4 and DNA ligase IV (Lig4) are the most specific C-NHEJ factors and required for V(D)J recombination and a normal level of CSR [60,61]. XRCC4 cooperates with Lig4 to catalyze the ligation step of C-NHEJ [62]. Extensive studies performed in the past convincingly demonstrate an important role of RAGs in generating chromosomal translocations [52–54,63]. RAGs can mediate translocations through cryptic recombination signal sequences (RSSs) mechanism [52,53]. It has been shown that translocation partners of antigen receptor loci such as LMO2, SIL and SCL possess cryptic RSSs that are misrecognized and cut by RAGs [64–66]. In addition, RAGs can generate DSBs at altered DNA structures such as non-B DNA structures [63,67,68].

The effectiveness of adaptive immunity appears to come at a high price, especially in the case of B cells, since more than 90% of lymphomas are B cell derived [69]. This may not be surprising given that B cells undergo both V(D)J recombination and CSR, and DSBs arising in either process, if not properly repaired, may initiate chromosomal translocations [50]. Consistent with this notion, human and mouse B cell lymphomas often harbor clonal translocations linking oncogenes, such as *c-myc*, to *IgH*, *Igκ* or *Igλ* [36]. Collaboration between these processes has also been proposed to initiate translocations [70]. Many oncogenic translocations in mature B lymphomas occur during attempted CSR and involve AID-initiated breaks [50,71–74]; for example, the breakpoints of *Igh-c-myc* translocations identified in the CXP lymphomas often fall within or around S regions where AID-initiated DSBs cluster [41]. However, some of the translocations

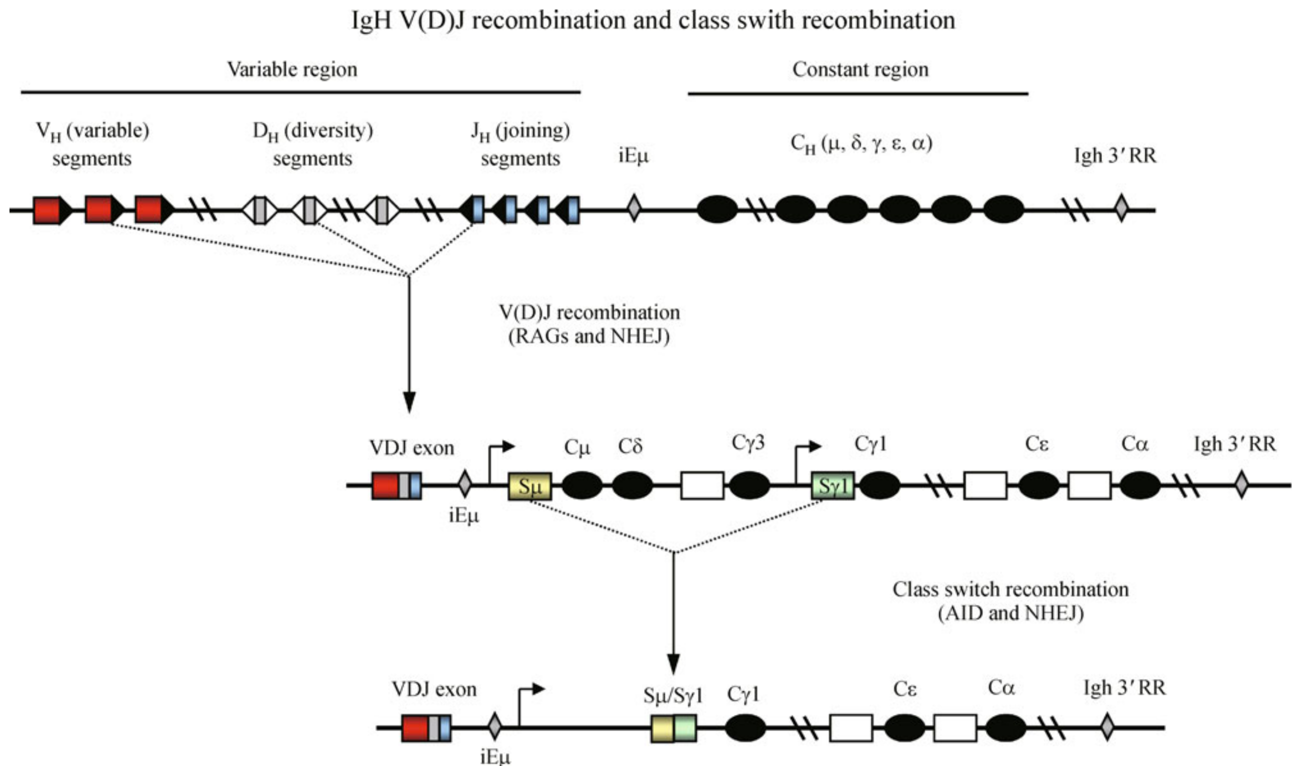

Fig. 1 Overview of V(D)J recombination and *Igh* CSR in B cells. Germline configuration of *Igh* locus (top) is shown with V (variable) (red box), D (diversity) (gray box) and J (joining) (blue box) gene segments located upstream and a set of constant region exons (black oval) located downstream. Grey diamond: intronic enhancer (iEμ) and Igh 3′ regulatory region (Igh3′RR). Black and white triangles flanking the V, D, J gene segments: recombination signal sequences (RSSs). V(D)J recombination is initiated by RAGs which recognize RSSs and completed by NHEJ. The rearranged *Igh* locus (middle) is shown with V(D)J exon assembled. White and color boxes: switch (S) regions. The upstream donor Sμ (yellow box) and downstream acceptor Sγ1 (green box), as an example, are indicated for CSR. CSR is catalyzed by AID and NHEJ. The switched *Igh* locus (bottom) is shown with the hybrid Sμ/Sγ1 sequence and the Cγ1 exons juxtaposed next to assembled V(D)J exon.

appear to result from RAG-initiated DSBs because the breakpoints often occur at D or J gene segments [36,52,75]. While many of these translocations probably indeed occur in B cell progenitors in BM during V(D)J recombination, our data suggest that some of these translocations may result from RAG activity in peripheral B cells [76]. Furthermore, we found that these RAG-initiated *Igλ* breaks often joined together with AID-initiated *Igh* breaks to form *Igh-Igλ* translocations in primary non-transformed peripheral B cells [76]. The frequency of such spontaneous translocations is so high that they can be readily observed using routine cytogenetic assay without employing any selective markers such as drug-resistant gene cassette, suggesting that frequency of DSBs might be a major driver of translocations.

Factors influencing the frequency and spectrum of translocations

Translocations require DSBs located on heterologous chromosomes. DSBs can be induced by cell-intrinsic factors such as oxidative metabolism, replication stress or lymphocyte specific recombination [54], or cell-extrinsic factors such as ionizing radiation (IR), which is still used experimentally to generate translocations, or chemotherapeutics. Mammalian cells are not tolerant of DSBs, which cause cells to arrest in mitosis or undergo apoptosis [77]. Thus, DSBs activate the cellular DNA repair machinery that catalyzes the joining of broken chromosomal ends [62]. There are two major pathways in mammalian cells, homologous recombination (HR) and NHEJ, to repair DSBs [62]. Joining of a single DSB with two broken ends on one chromosome is mediated by end-joining and could lead to deletions, duplications, or inversions, depending on the extent of the processing of the particular DSB. Joining of two DSBs on two heterologous chromosomes leads to translocations. Sometimes, the four broken chromosome ends on two chromosomes are almost precisely exchanged, which results in balanced reciprocal translocations with little nucleotides lost during the joining process. This type of translocations is often observed in mature B cell lymphomas [36]. In contrast, radiation-induced

translocations typically present an extremely complicated karyotype with multiple chromosomes joined together in one aberrant chromosome [78]. Thus, multiple factors could influence the frequency and spectrum of translocations, including: (1) the mechanism initiating DSBs; (2) the frequency of DSBs in a particular locus; (3) the spatial proximity of two heterologous loci harboring DSBs; (4) the repair pathway that fails to promote normal re-joining, instead, may prefer mis-joining. Apart from the influence of these mechanistic factors, oncogenic selection is also one of the most important factors to determine the appearance of recurrent translocations in cancers [51].

An elegant genetic system using embryonic stem (ES) cells was established to investigate the relationship between the frequency of DSBs and translocation [79]. The system took advantage of the unique features of meganuclease I-SceI, present in the mitochondria of *Saccharomyces cerevisiae*. I-SceI is an intron-encoded homing endonuclease and recognizes an 18 base pair sequence [80]. I-SceI is a rare cutting endonuclease because, statistically, an 18 bp sequence occurs once in every 7×10^{10} base pairs (equal to about 20 human genomes) [81]. Thus, it provides an ideal way to induce a single defined DSB in mammalian genome. In this ES cell system, two I-SceI cutting sites were targeted into two different loci located on Chr 14 and Chr 17, respectively [79]. The re-joining of DNA sequence between these two loci re-creates a selection marker (neo^r), which allows the detection of translocations formed [79]. The initial studies employing I-SceI system suggested that the presence of DSBs might influence the translocation pattern [79].

Subsequently, the I-SceI system was introduced into B lymphocytes to investigate how DSBs at *Igh* locus were repaired during CSR [82] and how DSBs at *c-myc* locus were generated [74]. More recently, the I-SceI-based experimental system has provided a tool to study translocation formation genome-wide, reported side-by-side from Alt's and Nussenzweig's groups [83,84]. Prior to these studies, most studies of translocation mechanisms focused on a few specific translocations that are observed as recurrent translocations in cancers [51]. However, cancer models may not be the ideal system to study the mechanistic factors influencing the early phase of translocation generation, given the strong influence of *in vivo* oncogenic selection pressure [51]. Previous studies using primary B cells, A-MuLV transformed pro-B cell lines, ES cell lines, and prostate cancer cell lines [76,85–87] have offered insights for the operation of mechanistic factors, also provided some clues for a better experimental system, such as minimizing cellular selection and employing genomic approaches [51].

In the Alt's study [83], a high-throughput, genome-wide translocation sequencing (HTGTS) approach was developed and employed to identify nearly 150 000 independent translocation junctions in B lymphocytes. These B cells harbor I-SceI cutting sites in *c-myc* or *Igh* locus, which were subsequently cut with retroviral I-SceI or I-SceI-glucocorticoid receptor fusion protein targeted into *Rosa26* locus [83]. Translocation junctions initiated from I-SceI DSBs in either *Igh* or *c-myc* locus were found to be widely distributed across the genome. Furthermore, the location of translocation junctions clustered to transcribed chromosomal regions and displayed a prominent correlation with transcription start sites [83]. Translocation hotspots mainly fall into two categories, AID-dependent or AID-independent. The former is consistent with the on-going AID-dependent CSR and the presence of AID-off targets in these anti-CD40 and IL-4 activated B cells while the latter comprises mainly cryptic I-SceI cutting sites [83]. This is probably because I-SceI is a homing endonuclease that does not have stringently defined recognition sequences in the way that other restriction enzymes do, which means single base changes do not abolish cleavage but reduce its efficiency to variable degrees [88]. Since the precise boundary of required bases is generally not known for homing endonucleases, the identified cryptic I-SceI sites may provide more insights into the functionality of I-SceI enzyme. Since the translocation libraries were sequenced with Roche-454 platform, the actual junctions were identified and majority of them were formed via end-joining with short micro-homologies [83]. In the Nussenzweig's study, a translocation capture sequencing (TC-Seq) method was developed to identify more than 180 000 chromosomal rearrangements genome-wide in primary B cells [84]. Basically, a pair-end deep sequencing approach using Illumina platform was employed to sequence the amplified rearrangements from LPS and IL-4 stimulated primary B cells that harbor I-SceI cutting sites in *c-myc* or *Igh* locus [84]. This study revealed that DSBs and transcriptional activity are critical factors to determine the pattern of chromosomal rearrangements [84]. Taken together, both studies suggest that the mechanism initiating DSBs probably plays a critical role in determining the frequency and spectrum of translocations. In particular, the translocation hotspots are often AID targets or cryptic I-SceI cutting sites in B cells that are activated for CSR and infected with retroviral I-SceI enzyme [83]. In addition, both studies show that the partners of recurrent translocations frequently identified in lymphomas also appear as translocation hotspots in the absence of major selections [83,84].

The role of spatial proximity in chromosomal rearrangements and translocations

To join the two broken chromosomal ends to form translocations, the loci with DNA lesions must be in close contact at some stage during the joining process. A long-lasting debate in the field of chromosome aberrations is whether these DNA lesions come into close contact after DNA damage (the "breakage first" hypothesis), or whether

rearrangements occur only where close associations pre-exist (the "contact first" hypothesis) [89]. The early dominance of breakage-first hypothesis likely stemmed from a traditional picture of chromosome architecture and interphase nuclear organization [89]. In the traditional view, chromosomes were envisaged as having a solid backbone that was completely severed by DSBs. These breaks then formed open mobile ends that wandered around in the nucleus, and rejoined with similar ends in the vicinity [90]. However, nowadays we know that chromosome integrity is maintained with DNA packaged with histone proteins into a complex tertiary structure [91]. Different chromosomes occupy distinct nuclear territories [91,92] and are further organized into open and closed chromatin domains within different nuclear compartments [93,94]. Thus, in this highly compartmentalized organization of the nucleus, it is almost impossible for unrestricted movements of open broken ends of DNA in a short period of time. It was predicted that most of the chromosomes are susceptible only to rearrangements within a domain (intrachromosomal) whereas very few rearrangements between chromosome domains (interchromosomal) would occur since most of the DSBs are not available [89]. Though, previous reports showed that DSBs could move around in the nucleus of yeast in a range of a few μm [95]. In addition, two recent studies followed the movements of fluorescent protein marked chromosomes that harbor I-SceI induced DSBs in live budding yeast, and showed that the damaged chromosomes increased their movements to search for homologous template for DNA repair [96,97]. Brownian motion of chromatin over distances up to 0.5–1 μm was also described in living cells of *Saccharomyces cerevisiae* and *Drosophila melanogaster* [98]. However, live-cell imaging studies of mammalian cells showed DSBs to be relatively immobile [99]. Thus, a more comprehensive analysis of chromosomal broken ends in live mammalian cells may be needed to further elucidate this point.

The highly organized structures of chromosomes have led to the notion that spatial proximity of two heterologous loci may promote their preferential translocations [100]. Consistently, cytogenetic studies showed several genes that involved in translocations were, on average, in relatively close proximity (see Refs. 76,101–103). However, such cytogenetic studies were limited by the experimental approach, for example, only a few pairs of genomic loci in a limited number of cells were analyzed. In addition, the definition of spatial proximity has been somewhat arbitrary in these cytogenetic studies [76,85,101,104,105]. Therefore, it is an important question to test how the three-dimensional (3D) organization of the genome contributes to frequency and spectrum of chromosomal translocations using high throughput genomic approaches.

Recently, a novel method was described, termed Hi-C, which probes the 3D structure of the genome by coupling proximity-based ligation with massively parallel sequencing [94]. By combining HTGTS and Hi-C, two high-throughput genomic methods, in a genetically tractable system, the first comprehensive analysis of 3D genome structure and the landscape of potential translocations within the same genome was revealed [106]. This study shows definitively that 3D genome organization and spatial proximity among loci strongly influence patterns of chromosomal rearrangements and translocations genome-wide [106]. Translocation libraries were generated from three G1-arrested A-MuLV transformed mouse $ATM^{-/-}$ pro-B cell lines; each of them harbored a single I-SceI cutting site integrated into different chromosomes, thereby, allowing generation of tractable I-SceI-induced DSBs at distinct genomic loci [106]. In this pro-B cell system, the most frequent translocation partners for I-SceI-induced DSBs were endogenous DSBs generated by RAG at the antigen receptor loci such as *Igκ* [106]. These results were consistently observed in all three pro-B cell lines, thus, suggesting proximity is not an important determinant factor in translocation formation under these conditions [106]. These data demonstrate that the frequency of DSBs is one of the drivers of translocation formation, consistent with the suggestions of previous finding [76]. To normalize the formation of DSBs in the genome, the G1-arrested pro-B cell lines were treated with IR, a situation in which DSBs are not rate-limiting anymore. In this scenario, translocation spectrum was not dominated by RAG targets, instead, translocation junctions were widely spread across different chromosomes and subchromosomal domains in a fashion directly correlated to pre-existing contact [106]. Notably, translocation junctions occurred preferentially *in cis* along single chromosomes, especially around the site where the defined I-SceI-induced DSBs were generated [106]. Similar findings were also reported in another independent study [107]. It was found that, in the absence of recurrent DNA damage such as AID or RAG-initiated DSBs, translocations between *Igh* or *c-myc* and all other genes are directly related to the frequency of their pre-existing contact [107].

These new findings may be relevant to the translocation formation in cancers. In lymphoid cancers, as mentioned above, RAG- or AID-initiated DSBs at antigen-receptor loci dominate the spectrum of translocations [50]. However, the DSBs at translocation partner loci in lymphoid cells or the DSBs in non-lymphoid cells probably occur at low frequency; in this scenario, factors other than DSB frequency, such as spatial proximity, may play a much more important role than in lymphoid cancers [85,104]. Accordingly, formation of translocations between randomly generated DSBs, such as those induced by chemo- and radio-therapies, will likely be influenced by spatial proximity, similar to what was observed in the IR-treated pro-B cell lines [106]. Furthermore, these results obtained using the high-throughput genomic approaches will help the interpretation of cancer genome sequencing data and the evolution of increasingly complex karyotypes in cancers [5,108–110].

Why chromosomal translocation matters? Its application to clinical medicine

Since their discovery, chromosomal translocations have made a critical impact on diagnosis, prognosis and treatment of cancers [2]. It has been long recognized that particular chromosomal translocations are often associated with subtypes of leukemia and lymphoma, which convinced hematologists and pathologists that chromosomal abnormalities, especially translocations, were a crucial etiological component of these diseases [2]. The development of cytogenetic techniques such as fluorescence *in situ* hybridization (FISH) and spectral karyotyping (SKY) have greatly helped to identify chromosomal abnormalities in cancer cells [111,112]. These techniques have been widely used in clinics to facilitate the diagnosis of cancers, especially for leukemia and lymphomas [2,4]. SKY is particularly useful in this context, because cancer cells often have multiple chromosomal rearrangements [111]. In addition, with the application of molecular biology techniques, chromosomal translocations can also be detected with polymerase chain reaction (PCR), reverse transcriptase PCR (RT-PCR) or multiplex RT-PCR, which allows patient samples to be screened for different translocations in parallel [2]. Given the rapid development of next generation sequencing techniques, its application in clinics might be sooner than what we expect and potentially make a profound impact in personalized medicine [113].

Apart from its application in diagnosis, clinicians discovered that the chromosomal abnormalities appeared to be very useful prognostic markers, as more cytogenetic analyses were collected from patients [4]. Now, many clinics routinely carry out karyotyping of leukemia or lymphoma cells before treatment since the presence of particular chromosomal aberrations seem to be a very useful indicator for patients' prognosis and responses to a certain type of treatment. For example, in ALL patients, the presence of t(12;21) translocation indicates a good prognosis, whereas the presence of the Ph chromosome with t(9;22) translocation often associates with rapid advancement of the disease [2]. More importantly, the distinct type of translocation is critical in determining the appropriate treatment. For example, an acute promyelocytic leukemia (PML) patient that harbors the t(15;17) translocation, fusing a part of the PML gene to the retinoic acid receptor α (RARA) gene to encode PML-RARA, is very likely to respond to all-trans retinoic acid therapy [114–116]. In addition, a CML patient that carries t(9;22) translocation can be treated with STI-571, which induces remissions in the vast majority of patients [32]. As discussed above, the success story of Ph chromosome has established a paradigm that may be followed for other recurrent chromosomal structural changes associated with human cancers. One of such example is the anaplastic lymphoma kinase (ALK) translocation in non-small cell lung cancer (NSCLC). In 2007, a group led by Hiroyuki Mano discovered a fusion gene, EML4-ALK, in a lung cancer patient and later in five others [16,17]. Such translocation activates ALK expression [16]. ALK is an oncogene that induces cell transformation *in vitro* and *in vivo*, which was first identified in 1994 as part of another translocation in anaplastic large-cell T lymphoma [117,118]. In addition, about 12% of neuroblastomas, a rare pediatric cancer, also exhibit ALK mutations [119]. EML4-ALK translocation is present in 2%–7% of NSCLC patients [120,121], and two other ALK fusions in lung cancer have been reported [122]. Among the 160 000 new cases of NSCLC each year in the US, at least 5 000 of them are ALK positive. A clinical trial of ALK inhibitor (crizotinib) in lung cancer has been recently completed, which appeared to have a positive effect on patients [120], and the FDA approval for crizotinib was subsequently granted in 2011. Overall, these studies again demonstrated the impact of basic research on the mechanistic study of translocations and treatment of cancers.

The frequency of chromosomal translocations and other aberrations among different populations may be influenced by environmental or genetic difference, which could serve as predictors of human cancer risk [123]. Epidemiology studies suggest that the relative frequency of specific subtypes of translocation could vary significantly among populations [124]. For example, the molecular structures of *Igh-c-myc* oncogenic translocation in BL differ dramatically according to the epidemiologic setting [125]. About 80% of chromosomal breakpoints in *Igh* locus involve the J_H region and 20% involve S regions in African BL; in contrast, more than 90% of breakpoints in *Igh* locus occur in S regions in European BL [125]. This observation is potentially caused by the high incidence of malaria transmission in African population [125]. Increasing epidemiologic evidence also suggest that MLL translocations in infant and childhood leukemia are associated with environmental factors such as maternal exposure to chemicals or defects of metabolic pathways [126]. These studies potentially have profound impacts on the prevention of translocation. Although the relative frequency of translocation in leukemia or lymphoma cases is well documented, the frequency of translocation (in the absence of disease diagnosis) among different populations remains largely unknown [124]. Future studies of translocation frequency in prospective cohorts should provide more insights into the natural history and mechanism of translocations and other leukemia initiating mutations.

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