Recurrent chromosomal translocations: Is proximity a rule?

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The role of recurrent chromosomal translocations in pathogenesis is well characterized in many leukemia subtypes; however, the factors leading to such preferential gene fusions are yet to be understood. The proximity of the genetic regions is considered important for genetic exchange, and interphase molecular cytogenetic methods can be employed to measure the same. The interphase genomic location of gene pairs taking part in translocations which are non-randomly associated with leukemia subtypes was studied for the extent of proximity by measuring relative distance and radial location. The FISH (Fluorescence In Situ Hybridization) signals corresponding to gene pairs were scored for relative distance and percentage of possible translocation pairs showing proximity which was found higher for BCR-ABL, PML-RARA and AML-ETO. The radial position of the gene pairs was also recorded to see if there is any preferred location in terms of nuclear centre or periphery for translocation partners. The results suggested no preferential location of any of the gene pairs in periphery or centre of the interphase nucleus, rather random distribution was observed for all the three cases. We report here the use of simple interphase FISH method to assess the interphase proximity of gene fusion pairs which can be further employed for other translocations.

Keywords: Chromosome territories, FISH, Genetic proximity, Interchromosomal translocation, Interphase genomic organization, Leukemia, Nuclear location of genes, Relative proximity

The progress in genomics in terms of sequencing has been significant however, the rules governing organisation and functional aspect of the spatial arrangement of chromosomes in interphase nucleus remains to be unravelled. Eukaryotic genome consists of chromosomes non-randomly positioned within the nuclear space in discrete regions or "chromosome territories". There are reports supporting well defined chromosome territories[1] and also the random arrangement[2]. The main focus of nuclear architecture studies now is to identify if there are non-random chromosomal positions in interphase nuclei, the possible mechanism of formation, and functional implications. However, the chromosome position is also reported to be tissue specific, hence any generalisation regarding role of spatial arrangement in phenomena like reciprocal exchange of genetic material is not simple.

The spatial organization of chromosomes plays an important role in gene regulation, genome stability maintenance, modulation of transcriptional activity through chromatin organization[3], and regulation of cellular and developmental pathways[4,5]. A number of chromosome instability diseases like cancer are also reported to be associated with altered spatial genome organization in interphase nuclei[6]. Specific chromosome positioning and proximity of territories are considered to be a mechanism that promotes interchromosomal translocation which leads to novel fusion transcript playing role in cancer pathogenesis[6,7].

The concept of territorial organization of interphase chromosomes in the animal cell nuclei was first mentioned by Carl Rabl[8]. The term "chromosome territory" (CT) was coined by Theodor Boveri for studies of blastomere stages of the horse roundworm *Parascaris equorum* or *Ascaris megalocephala* later and suggested that each chromosome maintains its individuality during interphase[9]. In 1950s it was suggested that chromosomes are present in the form of chromatin fibres of 10-30 nm in diameter which intermingle with each other like a bowl of spaghetti with no sign of individuality in interphase nuclei. The experimental evidence regarding chromosome territories was not obtained by many[10]. Hence, for a long time the random arrangement of chromosomes was a widely accepted assumption. With the advent of molecular cytogenetics and high end microscopy, it

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was possible to locate specific chromosome pairs in interphase[11]. The experiment by Thomas Cremer and Christopher Cremer, a cell biologist and a physicist demonstrated the interphase arrangement of chromosomes. A LASER beam was used to induce DNA damage focused within a small part of the cell nucleus. The radioactively labelled nucleotides were added that got incorporated during repair following DNA damage. The position of marked region was tracked by radiography during metaphase when cells entered next cell cycle. It was observed that very few immediately adjacent chromosomes were affected which supported the presence of chromosome territories that occupy discrete and limited volume in the nucleus. This experiment clearly demonstrated chromosome territory model rather than random organisation[12].

The chromosome territories constitute chromatin fibre loops of 30-150 kb, which in turn form rosettes to give the 1 Mb replication domains, or ~3 Mb giant loops that are formed by a random walk of the fibre and are held together at their bases[13]. An extensive network of channels generates a large surface and this makes regulatory factors easily accessible to the interior of the chromosome. During consecutive cell cycles the chromosome territory organisations constituted of replication domains of around 1Mb were maintained[14].

The positioning of chromosomes can be described in two ways; 'relative' i.e. mapping of preferred neighbour of a given chromosome; or 'radial' i.e. nuclear periphery or nuclear center. Bickmore and colleague introduced the concept of radial position in the study of arrangements of chromosome[15]. The relative positioning of chromosomes may be important in the formation of translocations[16]. It has also been suggested that clustering of genes in transcription hot spots contributes to their efficient regulation and expression[17]. The position of genetic loci and chromosome territories may have functional implications. There are reports regarding movement of several genes from a peripheral position into the interior upon their activation. Considering the fact that genes showed altered radial position without changes in expression, and that many genes do not undergo positional changes when their expression levels are modulated, indicates that radial positioning is functionally not tightly linked to gene activity[18]. It has also been reported that chromosomes occupy preferential relative positions within a cell nucleus but

that these positions are not strictly maintained in all cells of a population[19].

The study of factors determining arrangement of chromosome territories in interphase cell nuclei has been a focus of research. The chromosome paint FISH experiments in human lymphocyte nuclei revealed that gene density and size play a major role and each chromosome predominantly occupies its own micrometer-scale territory during interphase[20], and it was also observed that gene-dense and gene-poor chromosome territories exhibit different higher-order nuclear organization patterns[15,20,21] that vary as per the cell and tissue types, and can change during differentiation and development[20]. HSA19 (Human Chromosome) CTs (Chromosome territories) are known to have highest gene density and are consistently found in the interior in case of human lymphocyte nuclei and numerous other cell types, whereas the territories of the gene poor HSA 18 were at the nuclear periphery[15,20,22].

In addition to tissue specificity, the species specific variation has also been reported. In BALB/c mouse 90% cases show t(12;15) whereas in BALB/cRb6.15 mouse it was t(6;15). The 3D-FISH (Three-dimensional fluorescence in situ hybridization) analysis revealed that chromosomes 6 and 15 were present in close proximity in BALB/cRb6.15 mice, but not in BALB/c mice[23].

Along with all this large scale correlations there occurs large scale randomization, however the significance of such processes are still not clear[4,24-26]. In 2002, Michael tried to study randomness within human interphase nuclei with 24-colour whole-chromosome painting, after damaging the lymphocyte at interphase stage by sparsely ionizing radiation *in vitro*. A cluster of five chromosome pairs i.e. 1, 16, 17, 19, and 22 preferentially located near the center of the nucleus were found to be exception to randomness amongst all 22 autosomes[2]. The similar results were also previously reported by Boyle[21].

Chromosomal neighbourhood is known to affect the probability of translocations due to non-random positioning of participating chromosomes. Till date more than 600 recurrent balanced chromosomal rearrangements have been documented in human cancers, predominantly in leukemia[27]. For reciprocal translocation, two chromosomes must have a free end generated by a double-strand break in DNA concomitantly which should either be in close contact or should be brought into proximity[28]. Earlier studies

have suggested these free DNA ends can move within a wide range, up to 2 μm[26,29,30]. But according to more recent studies the end of a broken mammalian chromosome is shown to have very limited mobility which can move no more than 0.2 μm[31]. Thus, based on these studies the proximity is considered to be an important factor in events of rearrangements.

Analysis of the relative positions of BCR and ABL in haematopoietic cells revealed that these two loci are in close proximity more often than would be expected by chance[32,33]. Similarly, the genes PML and RARA, which are fused due to t(15; 17) translocation and play a role in pathogenesis of pro-myelocytic leukaemia, were often found to be close to each other[33]. The fact that distinct translocations are associated with different types of cancers might indicate that the pattern of chromosome proximities is distinct and specific for different tissues[34]. Recurrent chromosomal rearrangements are common in cancer cells and may be influenced by non-random close positioning of recombination-prone genetic loci in the nucleus. If proximity dictates the translocation, one might expect that the native position of two gene loci participating in translocation would be close to each other.

Materials and Methods

We hypothesise that the commonly reported gene loci participating in chromosome translocations will show proximity in the nucleus. To test this hypothesis, we selected three different cases of translocations; t(9;22) involving fusion of ABL and BCR genes; t(15;17) involving fusion of PML and RARA genes; and t(8;21) involving fusion of AML and ETO for the current study that are non-random and even diagnostic for the leukemic subtype.

The samples of leukemia patients with chronic myeloid leukemia, acute promyelocytic leukemia, and acute myeloblastic leukemia M2 type were studied for t(9;22), t(15;17) and t(8;21) respectively as the chromosome arrangement is reported to be tissue specific. The bone marrow smears analysed with FISH for gene pairs that are diagnostic for a leukemia sub-entity were selected for the analysis if they were negative for translocation due to cytogenetic remission. FISH technique enables the direct localisation of labelled DNA probe to study the organisation of chromosomes at interphase nuclei[35].

The commercially available fluorescent labelled complementary DNA probes were used for fluorescence in situ hybridization followed by DNA specific counter stain DAPI. The digital image capture and analysis was carried out using Carl Zeiss Microscope and ISIS software (Metasystems). The FISH signals for specific gene pairs were differentially labelled with green and orange fluorochromes, and cells showing two green and two orange signals separately were scored for relative and radial position of the gene loci as described above in order to categorize the gene pairs taking part in translocation as 'in proximity' or 'not in proximity' defined as under. The working definition for 'proximity' for the current study in terms of "relative distance" in the nucleus is as follows:

The distance of less than 30% of the nuclear diameter was considered as close proximity, and more than 30% as not in proximity for relative distance. The criteria of less than 20% of nuclear diameter have also been reported[19]. The distance between possible translocation partners involving one each from the four possible combinations was measured as depicted in Fig. 1A.

Whereas for "radial position", the signals for gene pairs were considered as 'central' when the position was within 50% of the radius from centre, and 'peripheral' when the position was more than 50% of the radius (Fig. 1B)[7]. With reference to radial position, the 'C' or 'P' locations of both the pairs of homologues were noted in all three translocations.

The present study was carried out on samples received and processed for diagnostic purpose only, hence informed consent was not necessary to obtain form the patients for the above work.

Results

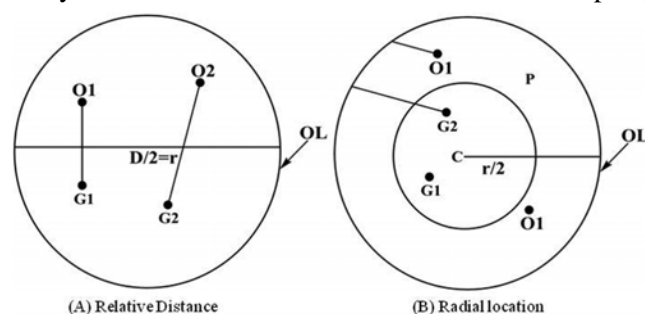The BCR-ABL genes specific FISH signals were analysed in 815 cells from 18 different samples;

Fig. 1—(A) Schematic diagram of measurements in terms of distance between potential translocation partners (a-d) (Relative Distance); and (B) Central or peripheral position of translocation partners (e-h), (i) C-Centre, (j) P-Periphery and D-diameter of interphase cell based on the FISH signals; where a) G1-O1, b) G1-O2, c) G2-O1, d) G2-O2, e) G1-OL, f) G2-OL, g) O1-OL, h) O2-OL]

PML-RARA genes specific FISH signals analysed in 563 cells from 13 different samples; and. the AML-ETO genes specific FISH signals analysed in 635 cells from 12 different samples. Table 1 depicts distribution pattern of homologues and translocation pairs for each of the three translocation cases.

Distance between translocation partners in terms of four possible combinations was measured for relative proximity; G1-O1 / G1-O2 / G2-O1 / G2-O2. Fig. 2 depicts the percentage of cells with or without proximity of possible translocation pairs. The observations are depicted in Table 2.

Radial location in terms of relation to centre or periphery was noted for both the pairs of homologues taking part in translocation depicted in Fig. 3.

Discussion

Positive correlation between spatial proximity of genetic loci in interphase nuclei and translocation

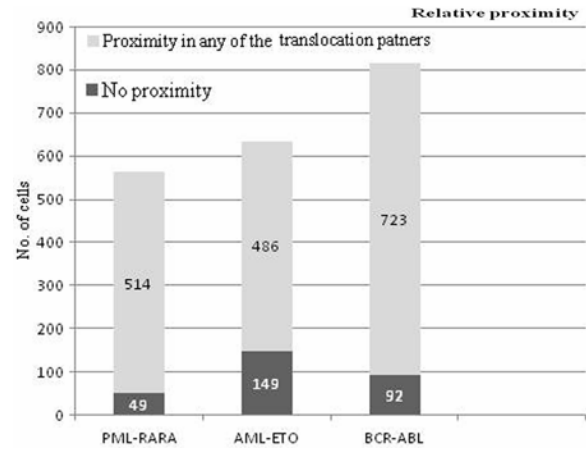Fig. 2—Results of relative proximity in three cases of translocation partners in terms of four possible combinations (G1-O1 / G1-O2 / G2-O1 / G2-O2)

Table 1—The radial position of (A) ABL (Green) & BCR (Orange); (B) PML (Green) & RARA (Orange); and (C) AML (Green) & ETO (Orange) signal pairs in interphase nuclei in leukemia bone marrow samples in cytogenetic remission.

Signal		In center	Pairs in centre	In periphery	Pairs in periphery
(A) ABL (Green) & BCR (Orange)*					
G1	G1&G2	362	180	453	236
G2		397		418	
O1	O1&O2	387	207	428	270
O2		365		450	
(B) PML (Green) & RARA (Orange)**					
G1	G1&G2	265	144	298	173
G2		269		294	
O1	O1&O2	301	185	265	135
O2		311		252	
(C) AML (Green) & ETO (Orange)***					
G1	G1&G2	200	72	435	291
G2		216		419	
O1	O1&O2	157	40	478	390
O2		128		507	

[* negative for t(9;22)(q34;q11), ** negative for (15;17)(q22;q21), and *** t(8;21)(q22;q22)]
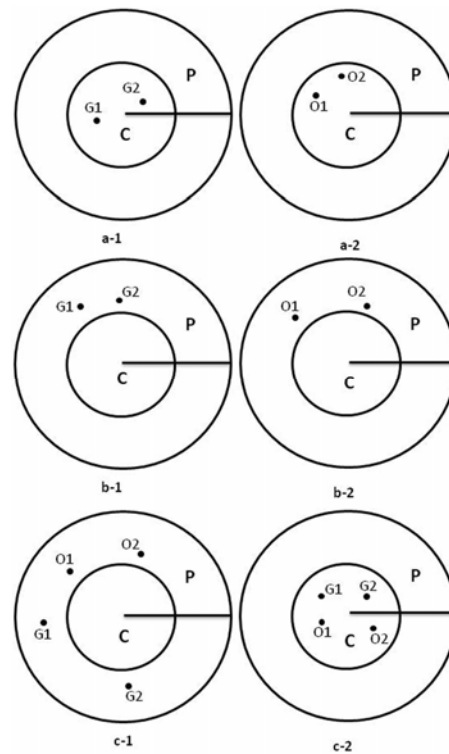

Fig. 3—Radial location of homologue pairs G1G2 (a-1) & O1O2 (a-2) in central region (within r/2 from center) & G1G2 (b-1) & O1O2 (b-2) in peripheral region (within r/2 from periphery) and both the pairs in periphery (c-1) center (c-2).

Table 2—The number of cells showing proximity in all four possible combination, no proximity in any combination and proximity in any combination out of total cell studied for each translocation

Translocations	Total cells	Proximity in all four possible combinations	No proximity in any of combination	Proximity in any one out of four combinations
t(9;22)(q34;q11)	815	73	92	723
t(15;17)(q22;q21)	563	71	49	514
t(8;21)(q22;q22)	635	19	149	486

Table 3—The percentage of cells showing relative and radial proximity of translocation pairs and homologous pairs

Translocation partners	Total cells	% cells in relative proximity	% pairs in centre		% pairs in periphery		% both pairs in centre	% both pairs in periphery
			G1&G2	O1&O2	G1&G2	O1&O2		
PML-RARA	563	91.29	25.57	32.85	30.72	23.97	8.88	9.23
AML-ETO	635	76.53	11.33	6.29	45.82	61.41	0.78	29.44
BCR-ABL	815	88.71	22.08	25.39	28.95	33.12	5.76	10.30

frequencies has been suggested where chromosomes located in proximity undergo translocation events more frequently than distantly located ones[20,36,37]. Our hypothesis of relation between proximity and translocation was tested on three widely reported translocations i.e. t(15;17)(q22;q21), t(8;21)(q22;q22) and t(9;22)(q34;q11) using relevant tissue in cytogenetic remission cases as, the translocation positive cases would have fusion and no measurements would be meaningful. We have measured the distance of native location between two translocation pairs in order to categorise proximity in terms of relative and radial. The results suggested proximity of two genes taking part in translocation present on nonhomologous chromosomes as per our working definition of proximity in terms of relative distance. Higher percentage of cells showed relative proximity in all the three translocation pairs; 88.7, 76.5 and 91.3% of cells in BCR-ABL, AML-ETO and PML-RARA, respectively (Table 3). This observation suggests that the relative proximity of two chromosomes can be one of the factors that promote exchange of genetic material between the two. When nuclear radial position of potential translocation pairs was examined, it was observed that 22.08%, 11.33% and 25.57% cells had homologues in centre and 28.95%, 45.82% and 30.72% cells had homologues in periphery for BCR-ABL, AML-ETO and PML-RARA respectively. Similarly in case of partner pair of homologues; 25.39, 6.29, 32.85% cells had the pair in centre and 33.12, 61.41 and 23.97% cells had the pair in periphery for BCR-ABL, AML-ETO and PML-RARA, respectively (Table 3). Thus indicating that while relative proximity was more frequent, radial location of any of the translocation pairs was not non-random or fixed.

In the current study, we have considered the relative distance between the gene-partners located on specific chromosomes assuming that each chromosome as a whole is in a territory. The 3D conformation of the individual chromosome in interphase can affect the proximity of the fusion gene partners, but it may be sufficient to record the position of the gene of interest rather than whole chromosome. A software for Image Analysis of Chromosomes for computing localization which can compute localization of whole chromosomes is also reported, the analysis showed that chromosome territories have non-random gene density based organization within the interphase nuclei of human fibroblasts[38].

Further studies involving more sensitive methods are necessary regarding spatial organisation of chromosome territories and role of proximity of genes or chromosomes in translocation for understanding mechanism of genetic alteration in certain diseases including congenital disorders and cancer.

Acknowledgement

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