Chapter 3

Acquired Chromosomal Abnormalities and Their Potential Formation Mechanisms in Solid Tumours

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Additional information is available at the end of the chapter

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Abstract

Solid tumours include numerous malign or relatively less benign types of carcinomas and sarcomas. Acquired chromosomal abnormalities in solid tumours are hallmarks of gene deregulation and genome instability. Chromosomal abnormalities are mainly classified into two groups: structural and numerical alterations. Structural rearrangements involve chromosomal aberrations such as deletion, translocation, duplication, inversion and gene amplification, whereas numerical abnormalities result in aneuploidy or polyploidy. Structural chromosome abnormalities can arise from non-allelic homologous recombination (NAHR), non-homologous end joining (NHEJ) and fork stalling and template switching (FoSTeS). Numerical abnormalities can form through various errors in the mitotic spindle checkpoint and some cellular processes during mitosis. This chapter reviews acquired structural and numerical chromosomal abnormalities in solid tumours and presents potential formation mechanisms. In this chapter, the relationship between long inverted repeats (LIRs) and *MYCN* amplification in neuroblastoma was also investigated. The distribution of LIRs was determined at chromosome 2p25.3–2p24.3, using inverted repeat finder (IRF) software. LIRs were also identified at boundaries of amplicons in 14 neuroblastoma cell lines and 42 solid tumours, involving *MYCN* amplification. Statistical analysis showed a significant association between LIRs and *MYCN* amplification loci. Present data provide important insights into *MYCN* amplification mechanism. Therefore, a new model mechanism for formation of the *MYCN* amplification is proposed at the end of the chapter.

Keywords: solid tumour, chromosomal abnormalities, model mechanisms, long inverted repeats (LIRs), neuroblastoma, *MYCN* amplification mechanism

1. Introduction

Acquired chromosomal abnormalities during clonal evolution of tumour cells, which can influence biological or clinical behaviour in a solid neoplasm, are hallmarks of gene deregulation and genome instability [1, 2]. Acquired clonal cytogenetic abnormalities have been reported in more than 50,000 cases (current total number of the cases: 66,675, updated in 2016) in all main cancer types [3, 4]. Secondary chromosomal aberrations that are considered important unbalanced changes acquired during tumour progression usually result in large-scale genomic imbalances, whereas primary balanced abnormalities can cause a disease-specific gene rearrangement in cancer initiation [3, 5].

Chromosomal abnormalities are mainly classified into two groups: structural and numerical alterations [6]. Gross structural rearrangements involve the chromosomal changes including deletion, translocation, duplication, inversion, and gene amplification, whereas numerical abnormalities lead to abnormal number of a whole chromosome or entire chromosome set, resulting in aneuploidy and polyploidy, respectively.

Solid tumours include various malign or relatively less benign cancer types observed in multiple solid organs, systems and tissues, involving many carcinomas and sarcomas such as thyroid adenocarcinoma and Ewing sarcoma or adenomas such as salivary gland adenoma, respectively, as summarised in **Table 1** [7]. The Atlas of Genetics and Cytogenetics in Oncology and Haematology represents a large number of chromosomal abnormalities including translocation, deletion and inversion reported in solid tumours [7]. Together, the Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer presents the recurrent structural and numerical chromosome abnormalities reported in at least two cases with the same morphology at any topography of the body in solid tumours (**Table 1**) [4].

Recurrent aberrations share a common size and consistently recur in different individuals, leading to clustering of the breakpoints, whereas the nonrecurrent rearrangements formed within a region are in different size in each patient, but these rearrangements may share a smallest region of overlap (SRO), which may cause similar clinical phenotypes [8]. Recurrent abnormalities mostly occur via non-allelic homologous recombination (NAHR) between low copy repeats (LCRs) [9]. Nonrecurrent rearrangements are usually explained by non-homologous end joining (NHEJ) and the fork stalling and template switching (FoSTeS) models [8].

The recurrent structural rearrangements and chromosomal gains that are present in at least two cells in a neoplasia are accepted as clonal; however, according to the International System for Human Cytogenetic Nomenclature (ISCN), missing chromosomes that are observed in at least three cells are accepted as clonal [10]. The chromosome abnormalities detected at a frequency of less than 5% in an examined cell population are considered non-recurrent or non-clonal [11].

The balanced structural chromosomal rearrangements involving mostly translocations and some inversions, such as t(12;16)(q13;p11) in myxoid liposarcoma and t(X;1)(p11;q21) in papillary renal cell carcinoma, are decidedly more disease specific than the unbalanced changes [12]. However, unbalanced structural alterations are more frequently observed than the balanced aberrations in solid tumours, see **Table 1** in Ref. [12].

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		Chromosomal abnormalities[b]				
		Structural				Numeric.
Site[a]	Solid tumour[a]	Trans.	Del	Dup	Inv	Aneup.
Bones	Ewing sarc. malign	t(11;22) (q24;q12)	del(22)(q12)	—	—	+8, +2 +12, −10
	Osteoblast. benign	—	—	—	—	+16, −13 −22
	Osteosarc. malign	der(1)t(1;3) (p36;p21)	del(1)(q11)	—	—	+7, +20 −13, −10
Digestive organs	Hepatoblast. malign	der(4)t(1;4) (q12;q34)	del(1)(p22) del(1)(q12)	—	—	+20, +2 +8, −18
Female organs	Ovary AC malign/benign	t(6;14) (q21;q24)	del(6)(q21) del(3)(q21) del(1)(q21)	dup(1) (q21q32)	inv(3) (p13p25)	+12, +3 −15, −X
	Breast AC malign	t(14;15) (p11;q11)	del(1)(p13) del(1)(p22)	dup(1) (q21;q44)	inv(1) (p22p36)	+1, +7 −X, −22
Head and neck	Larynx SCC malign	t(1;2) (p22;q21)	del(22)(q13) del(3)(p11) del(8)(p21)	—	—	+7, +20 −21, −Y
	Salivary gland Ad. benign	t(3;8) (p21;q12)	del(3)(p21) del(8)(p12)	—	inv(12) (q15q24)	+7, +8 −19, −Y
Lung heart skin	Myxoma benign	—	—	—	—	+7, −X −Y
	Malignant Melanoma Malign	t(1;14) (q21;q32)	del(9)(p21) del(6)(q13)	—	—	+7, +20 −10, −21
Male organs	Prostate AC malign	t(8;21) (q24;q22)	del(7)(q22) del(10)(q24)	dup(7) (q22q32)	—	+7, +Y −8, −Y
	Testis teratoma benign	der(1)t(1;14) (p11;q11)	del(6)(q21) del(1)(p35)	—	—	+8, +21 −13, −18
Nervous system	Glioblast. malign	der(1)t(1;12) (p36;q13)	del(9)(p21) del(9)(p13)	dup(1) (p11p36)	inv(19) (p13q13)	+7, +20 −10, −Y
Neuro-endoc./ endoc. system	Thyroid AC malign	t(2;3) (q13;p25)	del(12)(p11)	—	inv(10) (q11q21)	+7, +20 −22, −Y
	Pituitary AD.	—	—	—	—	+7, +12 −21, −22
	Benign neuroblast. malign	der(1)t(1;17) (p32;q21)	del(1)(p22)	—	inv(2) (p13p23)	+7, +17 −19, −X
Soft tissues	Alveolar rhabdo-myosarc. malign	t(2;13) (q35;q14)	del(13)(q14) del(16)(q22)	—	—	+2, +20 −3, −10
	Synovial Sarc. Malign	t(X;18) (p11;q11)	del(3)(p21) del(11) (q13q21)	—	—	+8, +12 −3, −14

		Chromosomal abnormalities[b]				
		Structural				Numeric.
Site[a]	Solid tumour[a]	Trans.	Del	Dup	Inv	Aneup.
Urinary system	Kidney AC malign	der(3)t(3;5) (p13;q22) t(X;1) (p11;q21)	del(3)(p14) del(3)(p13)	—	inv(1) (p36q21)	+7, +16 −14, −Y
	Wilms tumour malign	t(2;14) (q21;q24)	del(1)(p13) del(11) (p13p14)	—	—	+8, +12 −16, −22

[a]Information regarding solid tumours and their sites was obtained from database: 'Atlas of Genetics and Cytogenetics in Oncology and Haematology' [7].

[b]Chromosomal abnormalities were selected among the recurrent aberrations that are reported at most cases in the 'Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer' [4].

Abbreviations: AC, adenocarcinoma; AD, adenoma; Aneup., aneuploidy; Endoc., endocrine; Glioblast., glioblastoma; Hepatoblast., hepatoblastoma; Neuroblast., neuroblastoma; Numeric., numerical; Osteoblast., osteoblastoma; Sarc., sarcoma; SCC, squamous cell carcinoma; Trans., translocation.

Table 1. Common chromosomal abnormalities in solid tumours.

Aneuploidy (91.1%), an abnormal chromosome number deviated from euploid, is a very common feature in solid tumours, see **Table 1** in Ref. [13]. Aneuploidy is one of the main resultants of chromosomal instability and probably contributes to tumourigenesis through genomic variation and gene/protein dosage changes [14].

Gene amplifications are predominantly observed in solid tumours, as compared with haematological malignancies and lymphomas; see **Table 1** in Ref. [15]. Oncogene amplification can play an important role in the progression of solid tumours. Genomic DNA amplifications lead to a selective increase in the dosage of cellular oncogenes, usually resulting in overexpression of those genes and thus may provide contribution to the tumourigenesis [16]. *MYCN* amplification is a poor prognostic factor in neuroblastoma [17]. In addition, gene amplification, which involves multiple genes such as *MDM2*, *EGFR*, *MYCN*, *CCND1* and *CDK4*, is associated with poor prognosis in anaplastic grade III oligodendrogliomas, regardless of the gene involved [18].

Cytogenetic analysis in solid tumours is performed in a limited number of mitoses due to low mitotic index of tumour tissues, problems in disaggregation of sample, and intense necrosis in the periphery. Moreover, most of metaphases obtained from tissue culture have insufficient quality for karyotype analysis [19]. The technological developments in conventional cytogenetic, molecular cytogenetic, and molecular biological methods increased the quality and number of mitoses, which enhance efficiency and accuracy of karyotype analysis in solid tumours [20–22], while in vivo experimental models provided important insights into the mechanisms of chromosomal abnormalities [23, 24]. However, the mechanisms of chromosome abnormalities remain to be completely clarified.

Primary model mechanisms proposed for formation of structural chromosome abnormalities in genomic disorders and cancer are NAHR, NHEJ, and FoSTeS [8]. NAHR events are mostly

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associated with LCRs, but any evidence for such a direct association of NHEJ and FoSTeS with a specific DNA element was not yet provided. However, it was proposed that FoSTeS may be stimulated by a palindrome or cruciform structure [8]. In addition, the location of multiple DNA elements showed significant associations with the breakpoint regions of in particular non-recurrent rearrangements. This subject was argued in non-recurrent rearrangements section of this chapter. The numerical chromosome abnormalities can arise from various errors in the mitotic spindle checkpoint and cellular processes such as kinetochore assembly, chromatid cohesion and centrosome replication, leading to missegregation of chromosomes during mitosis [25].

On the other side, multiple models involving replication and/or breakage-fusion-bridge (BFB) cycles for formation mechanism of gene amplification have been proposed. These mechanisms are clearly described in Section 3.1.7. *MYCN* amplicon units within amplification locus are often arranged as clustered head-to-tail tandem repeats in direct orientation, suggesting that *MYCN* amplification may be formed via a mechanism different than those involving BFB cycles that produce inverted arrangements [26]. Some replication-based mechanisms for *MYCN* amplification were proposed, see Section 3.1.7. This chapter reviews acquired structural and numerical chromosomal abnormalities and their potential formation mechanisms in solid tumours. Furthermore, in light of present data, the chapter proposes a new model mechanism for formation of *MYCN* amplification that is a poor prognostic factor in neuroblastoma.

2. Cancer and chromosomal abnormalities: past to present

The earliest known malignant neoplasm was diagnosed in skeleton of a resident lived in Mauer (Vienna, Austria) in the Neolithic period, around 4000 BC [27]. It was reported that this example exhibited the signs of multiple myeloma rather than a metastatic carcinoma. The word cancer comes from term karkinos, which was first used to describe a non-healing swelling or ulceration in a medical text, 'Hippocratic corpus', written in about fifth century BC [28]. Hippocrates also used the terms karkinoma and scirrhus to describe malignant nonhealing tumours and hard tumours, respectively. In addition, he recognised and described the nasal carcinoma, later proposed a treatment for this cancer.

Claudius Galenus, known as Galen of Pergamum, classified the tumours into three categories as onkoi (lumps or masses in general), karkinos (malignant ulcers), and karkinomas (nonulcerating cancers) in the second century AD [29]. He also distinguished the lumps and growths as benign and malignant types. Ibn Sina, known as Avicenna, addressed the esophagus cancer in Iran of the eleventh century [30]. He was the first physician to refer to this disease as cancer of the esophagus. A century after the cancer studies of Avicenna, the physician Ibn Zuhr or Avenzoar made the first clinical description of the polyploid colorectal tumour, uterine cancer, and basal cell carcinoma in his monumental treatise Al Taysir during the twelfth century [31]. In the sixteenth century, Gabriele Fallopius accurately described the clinical differences between benign and malignant tumours [29].

Gregor Mendel examined the offspring of hybrids after hybridization of pea plants and discovered the fundamental laws of inheritance in 1865 [32]. Charles Darwin developed the first comprehensive theory of heredity, based on the transmission of physical entities that are the basis of development through inheritance in 1868 [33]. Between 1874 and 1876, Walther Flemming described the stages of cell division in more detail and showed the transformation of fibrous scaffold and network within the nucleus into 'threads', resulting in two daughter cells [34]. Fleming decided that this fibrous scaffold and arrangement of nuclear threads were termed chromatin (stainable material) and mitosen in 1879 and 1882, respectively. Heinrich Wilhelm Waldeyer coined the term chromosomen (chromosome) for stainable bodies in 1888, after he observed the stainability of the nuclear 'threads' during division [34].

David von Hansemann was first person to describe aneuploidy in 1890 [35]. He observed abnormal mitotic figures in several carcinoma samples. These findings were later developed by Theodor Boveri. Boveri showed the unequal distribution of chromosomes to the daughter cells after the fertilization of sea-urchin eggs by two sperms between 1902 and 1914 [36]. He revealed that the chromosome is a unit of heredity and proposed that chromosomal aberrations caused the cancer. At the same time, Walter Sutton showed that chromosomes occurred in distinct pairs and segregated at meiosis in his study with grasshopper chromosomes [37]. Sutton was the first to point out that the chromosomes conformed to Mendel's heredity rules. In other words, Sutton and Bovery developed the first clear chromosome theory of heredity.

Nowell and Hungerford first showed that the chromosomal abnormality was associated with a specific cancer [38]. They discovered a minute chromosome known as Philadelphia (Ph) chromosome today in the neoplastic cells of cases with chronic granulocytic leukaemia in 1960.

Spriggs *et al*. reported that many solid tumours included the aneuploid cell clones, which are hiperdiploid and/or relatively less hipodiploid, harbouring chromosomes in varying numbers detectable even in same case in 1962 [39]. They suggested that the biological success of these aneuploid clones is due presumably to the natural selection of successful variants. Rowley detected the Philadelphia (Ph) chromosome in the bone marrow and a few blood samples from the patients with chronic myelogenous leukaemia (CML), using the quinacrine fluorescence and Giemsa staining techniques in 1973 [40]. Author also observed the second Ph chromosome in a case and trisomy 8 in two patients in blast crisis.

The homogeneously staining region (hsr) was first detected in drug-resistant Chinese hamster sublines and two neuroblastoma cell lines, SK-N-BE(2) and IMR-32, using the trypsin-Giemsa banding methods in 1976 [41]. The authors also showed that the hsr replicated relatively, rapidly and synchronously before the midpoint of the S phase. In addition, two identical giant marker chromosomes 1 (bearing der(1)t(1;17) translocation containing 2p24 hsr) including 1p deletion, in addition to intact 1, were identified in IMR-32 neuroblastoma cell line in 1977 [42].

Atkin and Baker revealed that the pericentric inversions involving the heterochromatic regions of the chromosomes 1 are relatively common in cancer patients including solid tumours in 1977 [43].

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The fluorescence *in situ* hybridization (FISH) method was developed by Bauman *et al*. in 1980 [44]. By *in situ* hybridization and Southern blotting methods, N-myc oncogene and its amplification in the hsrs were discovered in numerous neuroblastoma cell lines and a neuroblastoma tumour tissue by Schwab *et al*. in 1983 [45].

Comparative genomic hybridization (CGH) method was developed for detecting and mapping the relative DNA sequence copy number between genomes by Kallioniemi *et al*. in 1992 [46]. Multicolor spectral karyotyping (SKY), a molecular cytogenetic technique, for detecting and analyzing the chromosomal aberrations in clinical samples was developed by Schröck *et al*. in 1996 [47].

3. Chromosomal abnormalities

3.1. Structural chromosomal abnormalities

3.1.1. Recurrent genomic rearrangements

Recurrent structural genomic rearrangements often result from NAHR between LCRs in direct or inverted orientation [48]. NAHR involving nonallelic crossover is one of the homologous recombination mechanisms of two-ended double-strand break (DSB) repair and occurs in both meiotic and mitotic cells in human [49].

LCRs, also called segmental duplications (SDs), are region-specific DNA blocks of 10–400 kb in length with ≥97% identity between repeat copies [50]. SDs define hotspot of the chromosomal rearrangements and hence can act as mediator of normal variation or recurrent chromosomal aberrations associated with a genomic disease [51]. LCR-mediated NAHR mechanism occurs preferentially at the hotspots inside low copy repeats and yields the recurrent rearrangements with common size and clustered (fixed) breakpoints in unrelated individuals (**Figure 1a**) [8].

Ectopic interchromosomal and interchromatidal (intrachromosomal) recombination (NAHR) between directly oriented LCRs *in trans* can produce both deletion and duplication (**Figure 1b**), whereas intrachromatidal crossover *in cis* can result in only deletion [52]. Inversion can occur through ectopic crossing-over between inversely oriented LCRs *in cis* (**Figure 1c**). In addition, NAHR between inversely oriented LCRs on sister chromatids can cause an isochromosome formation [52]. NAHR between interchromosomal LCRs on nonhomologous chromosomes can lead to recurrent translocations [53]. Besides the segmental duplications, NAHR between interspersed repeats such as LINEs and Alus can result in de novo unbalanced translocation and interstitial deletion, respectively [54, 55].

NAHR hotspots, specific to meiosis, can cause *de novo* alterations in copy number of dosage-sensitive genes associated with some genomic disorders in germ line cells, resulting in structural rearrangements such as deletion and duplication [56]. NAHR also mediates the recurrent genomic rearrangements occurring at relatively high frequency in particular adults in human somatic cells, suggesting the accumulation of *de novo* variations after birth [57].

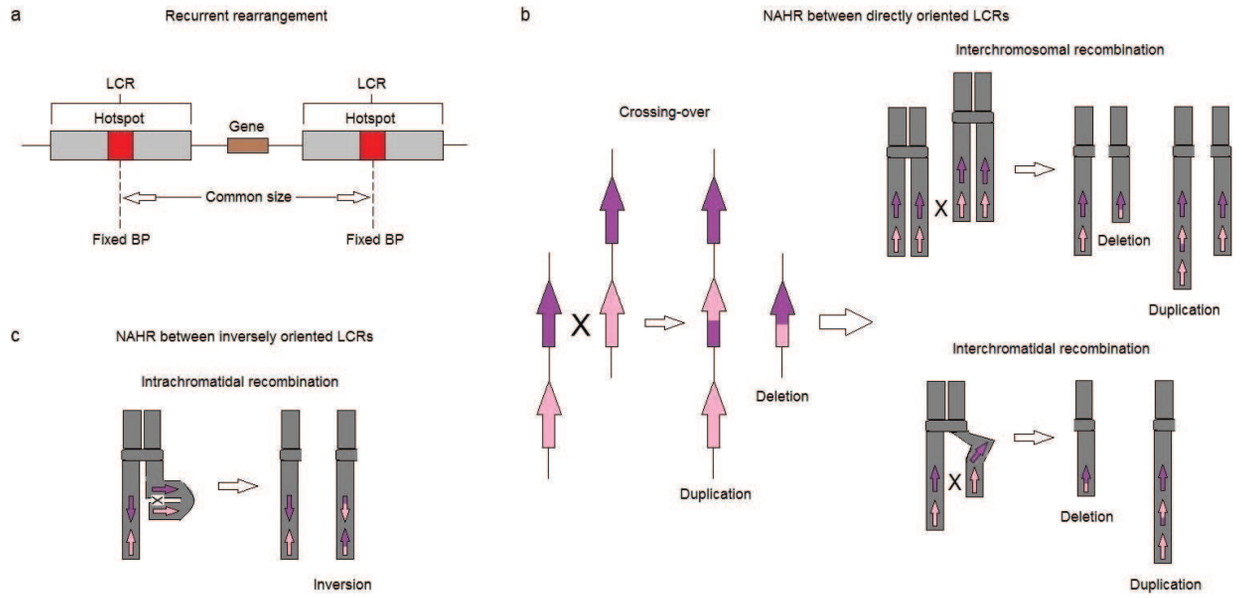

Figure 1. LCR-mediated nonallelic homologous recombination (NAHR). (**a**) Recurrent rearrangement with common size and clustered (fixed) breakpoints (BPs) resulting from NAHR between LCRs. (**b**) NAHR between directly oriented LCRs can yield both deletion and duplication through interchromosomal and interchromatidal (intrachromosomal) recombination. (**c**) NAHR between inversely oriented LCRs can result in an inversion through intrachromatidal recombination.

In addition, segmental duplications are markedly enriched at the multi-allelic CNVs, complex CNVs and loci including both deletion and duplication in human genome [58]. Carcinoma-associated breakpoint regions in human genome frequently contain SDs [59]. However, literature includes a limited number of the chromosomal abnormalities caused by NAHR mechanism in solid tumours. Of these studies, four reported that NAHR involved in large deletion of EXT1 and EXT2 genes in multiple osteochondromas and large deletion and duplication of NF1 gene in neurofibromatosis type 1 (NF1) [60–63].

3.1.2. Non-recurrent genomic rearrangements

Non-recurrent rearrangements are characterized by unique breakpoint junction in each individual but share an overlapped genomic region between the scattered breakpoints [8]. This SRO may encompass one or more genes (**Figure 2**), which are associated with a genetic disease or neoplasm. Due to the SRO region, the patients are likely to display similar clinical phenotypes. Like LCR in the recurrent rearrangement, any specific repeat causing a nonrecurrent rearrangement was not reported. However, one of the breakpoint locations of a nonrecurrent rearrangement in the genomic region can include relatively less scattered breakpoints in a smaller defined area, termed breakpoint grouping (**Figure 2**), suggesting that a genomic architecture such as palindrome or cruciform was extruded near this defined area [8].

The repetitive DNA sequence elements, such as inverted repeats, direct repeats, long inverted repeats (LIRs), *Alu* repeats, G-guadruplex-forming G-rich repeats and palindromic AT-rich repeats (PATRRs) were often detected in the breakpoint regions of many non-recurrent chromosomal abnormalities associated with genomic disorder, inherited disease or cancer in human [64–73].

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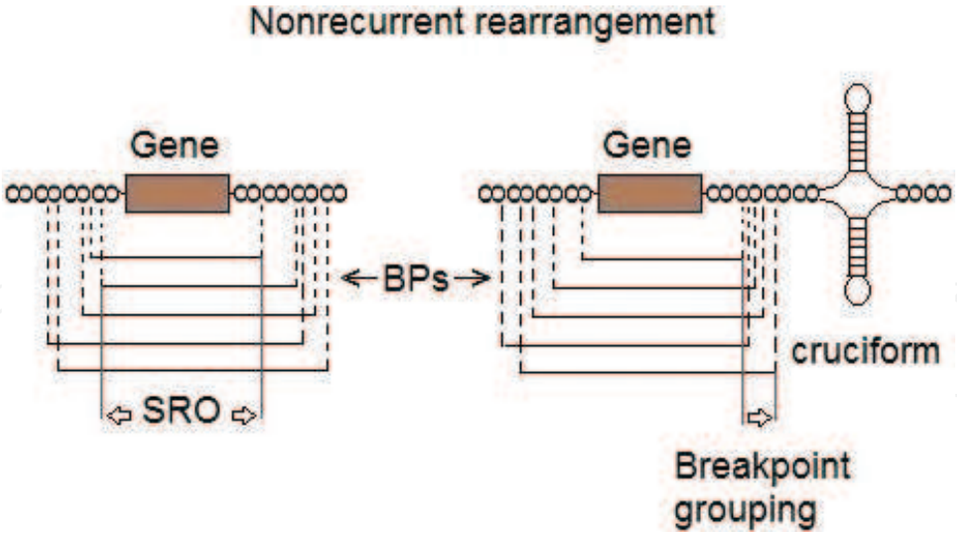

Figure 2. Nonrecurrent rearrangements share a smallest region of overlap (SRO). Dashed lines indicate the scattered breakpoints (BPs). In left side, a cruciform near the region containing the grouping of 3′ BPs is demonstrated.

Double strand breaks involving genomic rearrangements, translocations and deletions in neoplastic cells are usually joined by NHEJ [74]. NHEJ is active throughout the cell cycle, and its activity increases during transition from G1 to G2/M, whereas HR is most active in the S phase in human cells, concluding that normal human somatic cells also mostly utilised error-prone NHEJ at all cell cycle stages [75].

NHEJ mechanism tolerates nucleotide loss or addition at the rejoining site. This nonhomologous repair pathway requires three enzymatic activities (**Figure 3**), which involve the nucleases removing damaged DNA, the polymerases aiding in the repair and a ligase restoring the phosphodiester backbone [81].

Essential components of the canonical or classical NHEJ (c-NHEJ) include Ku70/80, DNA-PKcs and LIG4/XRCC4/XLF complex (**Figure 3a**), whereas the alternative forms of NHEJ, termed microhomology-mediated end joining (MMEJ), alt-NHEJ or A-EJ (**Figure 3b**, **c**), involves PARP1, MRN complex and its partner CtIP [77, 80, 82]. c-NHEJ actually plays a conservative role in genomic integrity but is versatile and adaptable in joining process of imperfect complementary DNA ends [83]. In other words, the accuracy of repair depends on the structure of DNA ends rather than c-NHEJ pathway [83].

A-EJ repairs the DSBs in the absence of key c-NHEJ proteins [84]. A-EJ is highly error-prone during end-joining process, leading to frequent DNA loss at the junctions and chromosomal rearrangements [79]. Other alternative end joining pathway, microhomology-mediated end joining (MMEJ), requires a microhomology of at least five nucleotides between DNA ends at the break sites and is independent of Ku70/80 and Ligase IV proteins of c-NHEJ but is dependent on MRN complex (Mre11, Rad50 and NBS1), Ligase III, XRCC1, FEN1 and PARP1 (**Figure 3b**), as compared to c-NHEJ that uses either no microhomology or sometimes terminal microhomology of 1–4 nucleotides between two ends [76, 78]. MMEJ can operate in where the microhomology is present, even in the presence of c-NHEJ in both cancer and normal cells [78].

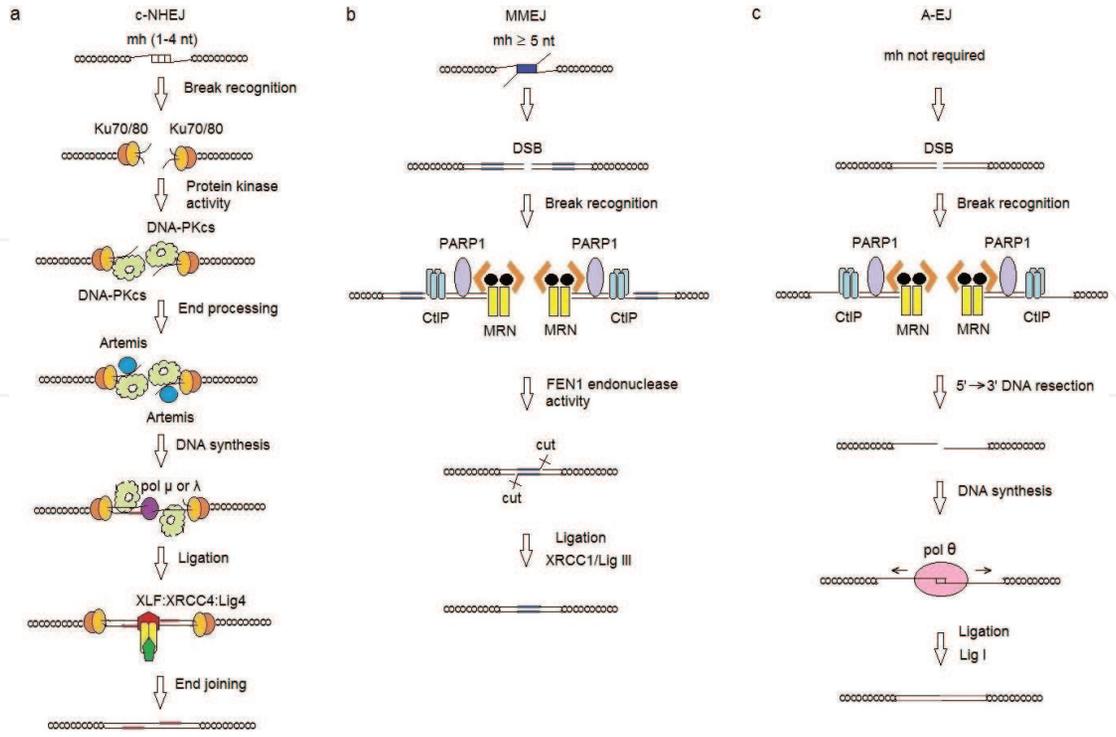

Figure 3. End joining mechanisms for repair of double-strand breaks. (**a**) Classical NHEJ (c-NHEJ) joins the DNA ends with microhomology (mh) of 1–4 nucleotides (nt) [76]. DNA break is recognised by Ku70/80, which recruits the DNA-dependent protein kinase catalytic subunit (DNA-PKcs). DNA-PKcs activates the Artemis that shows the endonuclease activity in both ends during end processing. DNA polymerase (pol μ or λ) performs the DNA synthesis, followed by ligation process (XLF:XRCC4:Lig4 complex) [77, 78]. (**b**) MMEJ joins the DNA ends with mh ≥5 nt. MRN complex and PARP1 recognise the DNA break, and CtIP starts the DNA resection. FEN1 endonuclease removes the flap, followed by ligation process with Ligase III (Lig III) [78, 79]. (**c**) A-EJ does not require the microhomology. A-EJ shares first step (break recognition) with MMEJ. But, A-EJ involves DNA synthesis with pol θ, followed by ligation with Lig I [79, 80].

In addition, replication-based mechanisms (**Figure 4**), FoSTeS and microhomology-mediated break-induced replication (MMBIR) for the formation of nonrecurrent rearrangements involving complex duplication and deletion, inversion, translocation, triplication and rolling circle were proposed [85, 86].

Taken together, the breakpoint analysis of structural chromosomal rearrangements in solid tumours shows that NHEJ, A-EJ and MMEJ are predominant mechanisms underlying these somatic aberrations; however, FoSTeS and MMBIR are responsible for a significant number of structural variations, in particular somatic complex deletions [87–89].

3.1.3. Translocations

Chromosomal translocations, which are one of the most common types of genetic rearrangements, generally arise from reciprocal exchange of heterologous chromosome fragments and can cause deregulation of gene expression through either juxtaposition of the oncogenes near promoter/enhancer elements or gene fusion [90]. Contrary to reciprocal translocations, Robertsonian translocations can be generated by joining between the long arms of two acrocentric chromosomes around a single centromeric region [91]. Reciprocal translocations can

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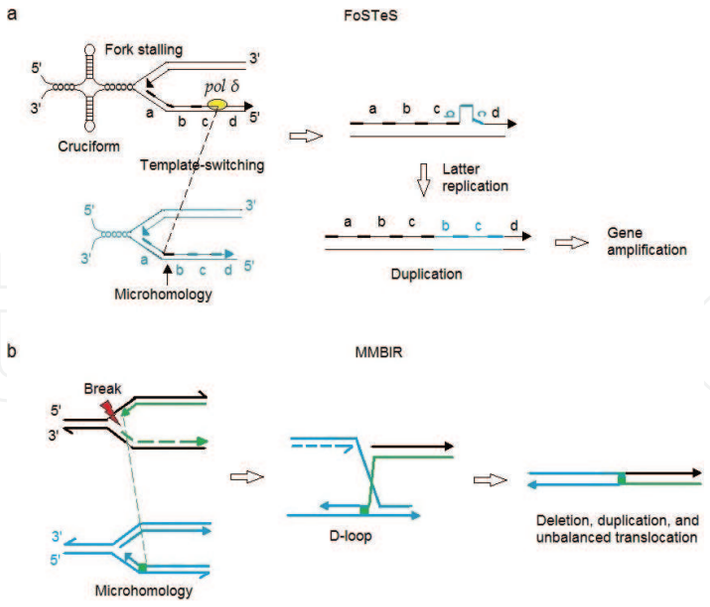

Figure 4. Replication-based mechanisms proposed for generating the nonrecurrent rearrangements. (**a**) A DNA lesion or a non-B DNA structure like cruciform can cause fork stalling during replication, stimulating the fork stalling and template switching (FoSTeS) mechanism [8, 85]. Lagging strand may then invade other fork via microhomology, leading to template-switching. By this way, duplication can arise after second round of replication. Successive template switching can result in gene amplification (see Section 3.1.7). (**b**) Microhomology-mediated break-induced replication (MMBIR) mechanism can be triggered by a single strand break, which gives rise to replication collapse [86]. The 3′ single strand overhang can invade a microhomology site at the other fork, and DNA polymerase restarts DNA synthesis through D-loop formation, resulting in deletion, duplication, and unbalanced translocation.

lead to balanced or unbalanced rearrangements. Balanced translocations do not cause the gain or loss of genetic material but can result in promoter swapping/substitution or loss of gene function, which was reported in some benign and malignant solid tumours [3], whereas unbalanced translocations result in a gain or loss, involving trisomy or monosomy in any chromosome segment, respectively [92].

Malignant solid tumours generally harbour non-recurrent balanced aberrations including many translocations rather than recurrent balanced ones; see **Table 1** in Ref. [3]. In addition, solid tumours show less often gene fusions compared to haematological disorders. Most of them, such as *EWSR1-POU5F1* (Bone sarcoma), *EWSR1-ERG* (Ewing sarcoma) and *PAX3-FOXO1A* (Rhabdomyosarcoma), were observed in sarcomas [3].

Many recurrent unbalanced translocations, such as der(1)t(1;1)(p36;q12) (Breast adenocarcinoma), der(3)t(3;6)(p11;p11) (Kidney adenocarcinoma), der(19)t(11;19)(q12;q13) (Lung squamous cell carcinoma) and der(12)t(11;12)(q12;q23) (Testis germ cell tumour), were detected in solid tumours [12].

Numerous inter-chromosomal and intra-chromosomal translocations in solid tumours were identified, and majority of these translocations were reported to form via NHEJ and A-EJ mechanisms (**Figure 5a**), while a small number of them was generated by FoSTeS/MMBIR [88]. A-EJ was more abundant in most cases. In addition, A-EJ is more significantly associated with breast tumours compared to other tumour types. Alt-NHEJ is also primary mediator of

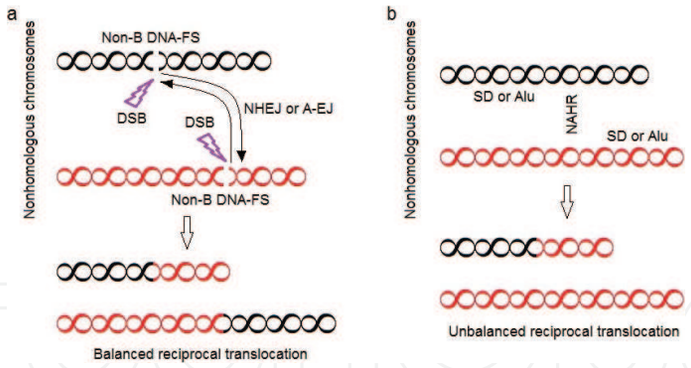

Figure 5. Reciprocal translocation mechanisms. (**a**) A balanced reciprocal translocation resulted from nonhomologous recombination. Random or a non-B DNA-forming sequence (Non-B DNA-FS) may induce the double-strand breaks on the nonhomologous chromosomes, stimulating NHEJ or A-EJ. (**b**) An unbalanced reciprocal translocation arose from nonallelic homologous recombination. Interchromosomal segmental duplications (SDs) or Alu interspersed elements may be mediator of NAHR [53–55, 93].

translocation formation in mammalian cells [94]. However, it was reported that c-NHEJ is the predominant mechanism for repair of the double-strand breaks, resulting in translocation formation in human cells [95].

On the other side, it was found that the breakpoint regions of recurrent translocations in solid tumours are flanked by segmental duplications and Alu element [93], suggesting that SD or Alu-mediated NAHR mechanism involves formation of recurrent translocations in solid tumours (**Figure 5b**).

In vertebrates, NHEJ is the main pathway for repair of DSBs, which is required for suppressing the chromosomal translocations [96]. However, the non-B DNA structures around breakpoint junctions can lead to chromosomal translocations (**Figure 5a**). Potential non-B DNA structure (e.g., hairpin/cruciform, triplex and quadruplex)-forming repeats such as inverted repeat, direct repeat, inversions of inverted repeat, (AT)n, (GAA)n, (GAAA)n, G4-DNA motifs and H-DNA are significantly associated with breakpoint regions of translocations in the cancers including solid tumours [97–99]. In addition, formation of *de novo* translocations between AT-rich repeats (PATRRs) was tested in cultured human cells. Contrary to *de novo* deletions, *de novo* translocations between PATRRs were not observed during both leading and lagging strand synthesis in the presence of slowed DNA replication. Kurahashi *et al*. thus proposed that translocation may be formed via a DNA replication-independent cruciform structure induced by PATRR [100].

3.1.4. Deletions

Chromosomal deletion is the most common structural aberration among recurrent unbalanced chromosomal abnormalities in solid tumours [12]. Chromosomal deletions are mainly classified into two groups as interstitial and terminal deletions. Interstitial deletion is formed by two breaks, whereas terminal deletion can occur due to one break near telomere [10].

Gross deletions can cause the loss of one or more genes in human-inherited diseases and cancers [71]. Heterozygous or homozygous deletions involving many tumour suppressor genes may play a major role in tumour initiation and progression. Interstitial heterozygous deletions within chromosome 3 common eliminated region I (C3CER I) including multiple genes such

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as *LIMD1*, *LTF* and *TMEM7*, mapped to 3p21.3, are widespread in solid tumours, suggesting that C3CER I region may harbour some tumour suppressor genes, besides its LOH may be causative in tumour development rather than reflection of an unstable genome in tumour cells [101]. Another study suggests that homozygous deletion of *PTEN* locus may be associated with metastasis in prostate cancer [102].

Homozygous deletions observed in multiple different chromosomal loci, some of them encompass LRP1B, FHIT, PARK2, CDKN2A (p16), CDKN2B (p15), PTEN, and WWOX tumour suppressor genes were frequently found in many cancer cell lines, usually derived from a solid tumour [103].

Many model mechanisms can explain gross genomic deletions. But, formation mechanism of deletion remains to be clearly enlightened.

NAHR-mediated deletion (**Figure 1**) was reported in a limited number of solid tumours. A study indicated that large deletion of EXT1 and EXT2 genes in multiple osteochondromas families can be occurred by NAHR between *Alu* repeats as well as NHEJ [60].

The genomic rearrangements including deletions in solid tumours are predominantly caused by end-joining mechanisms, NHEJ, MMEJ or A-EJ; however, complex deletions are generally formed by FoSTeS/MMBIR [87–89].

In addition, gross deletions have been associated with non-B DNA structure-forming sequences in breakpoint regions, including direct repeats, inverted repeats, inversion of inverted repeats, long inverted repeats (LIRs), and *Alus* in the genomes of cancers including solid tumours [71, 72, 97–99].

Gordenin *et al*. [104] previously proposed that an inverted repeat can form a hairpin at the lagging strand during replication, causing a deletion via slippage of DNA polymerase between short direct repeats adjacent to both side of stem of a LIR or between smaller repeats within LIR (**Figure 6a**). Lobachev *et al*. [105] proposed that homologous recombination between sister chromatids will repair the DNA strand without deletion at the inverted repeat site. According to their model, if another inverted repeat is present on the other chromosome, recombination then could lead to a deletion.

Later, it was shown that a hairpin formed by inverted repeat stalled the replication fork in both prokaryotes and eukaryotes, indicating that DNA hairpins are formed likely during lagging strand synthesis [106]. Kurahashi *et al*. [100] demonstrated that deletions occurred within PATRRs due to slow replication and uncoupling of DNA polymerase and helicase complex respectively during the synthesis of both lagging and leading strands in human cells, suggesting that replication slippage caused deletion of the hairpins induced by PATRRs in leading and lagging strand (**Figure 6b**).

Akgün *et al*. [107] proposed that the break generated by a nicking endonuclease in the top of hairpin can stimulate the cellular repair mechanisms, resulting in one-sided (in only one of repeat units) or two-sided (in both of the repeat units) palindrome deletions (**Figure 6c**). Cunningham *et al*. [108] showed that a nicking near hairpin tips by endonuclease in a perfect palindrome can result in deletions at the center of palindrome after rejoining of the breaks by NHEJ (**Figure 6c**).

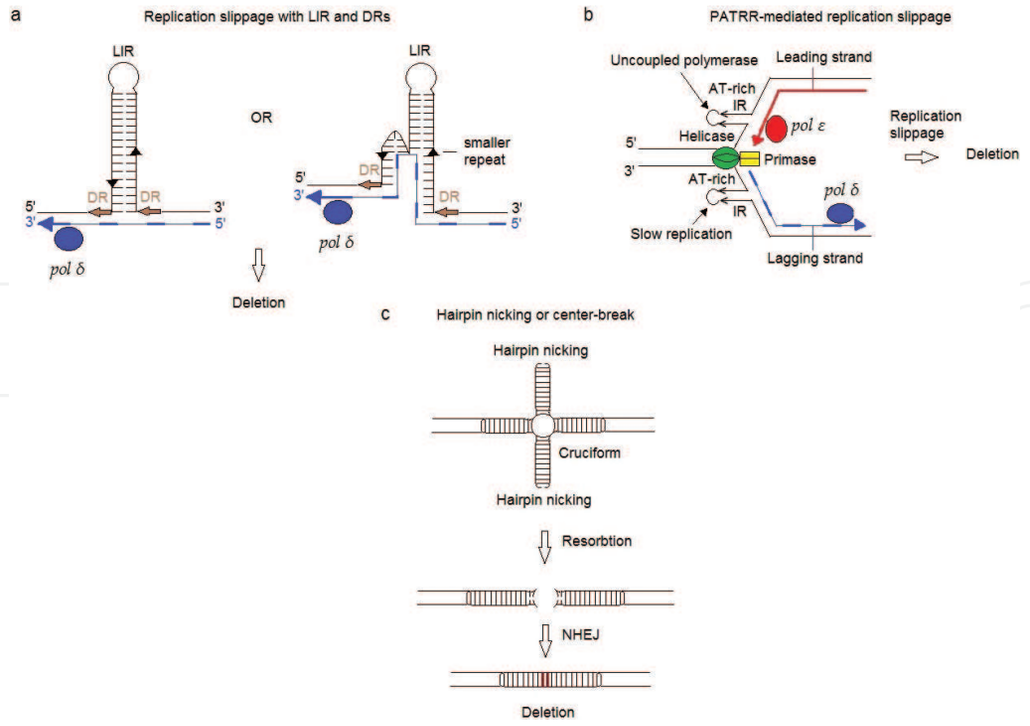

Figure 6. Chromosomal deletion mechanisms. (**a**) Replication slippage caused by a LIR in lagging strand during DNA replication. Direct repeats in both side of LIR and smaller repeats within LIR lead to deletions at entire LIR and a segment of LIR, respectively. (**b**) Replication slippage induced by palindromic AT-rich repeats (PATRRs). Deletion of both PATRRs in lagging and leading strand templates can form via slow replication and uncoupling of DNA polymerase and helicase complex, respectively. (**c**) Hairpin nicking or center-break mechanism. Hairpin nicking can result in deletion at the center or both sides of a cruciform. NHEJ rejoins double-strand break after resorbtion.

I previously found that LIRs are significantly associated with the breakpoint regions of gross deletions in human-inherited diseases and cancers [71]. Statistical analysis showed that a positive significant strong correlation was found between 5′ and 3′ LIR numbers. In addition, negative significant correlations were found between deletion size and the numbers of 5′ and 3′ LIRs. These results suggest that LIRs could be contributed to DNA sequence evolution in human genome. Statistical analyses also suggested that DNA strand is potentially broken in locations closer to bigger LIRs. Another analysis demonstrated that loop length and stem identity of 3′ LIRs were more important in larger deletions. In light of these findings, I proposed two model mechanisms involving LIR-mediated gene deletion (**Figure 7a**, **b**). In first mechanism, it was proposed that gross deletion can be generated by breaks formed near two LIRs at the 5′ and 3′ breakpoints, which are located two contiguous replication bubbles (**Figure 7a**). In second mechanism, it was proposed that back-folded stem loop structure can cause a second break at the 5′ breakpoint region after a break near 3′ LIR occurred during replication, resulting in gene deletion (**Figure 7b**). In this chapter, I also proposed a new modified model mechanism involving 5′ and 3′ LIRS within same replicon, adapted from other two ones (**Figure 7c**).

Hairpin structures were shown to form at an interrupted LIR with 111-bp stem and 24-bp spacer at the frequencies of 32–37% on both leading and lagging strand templates, respectively, suggesting that hairpins were extruded simultaneously by palindrome on both leading and lagging strand templates during replication [110]. However, another study showed that an

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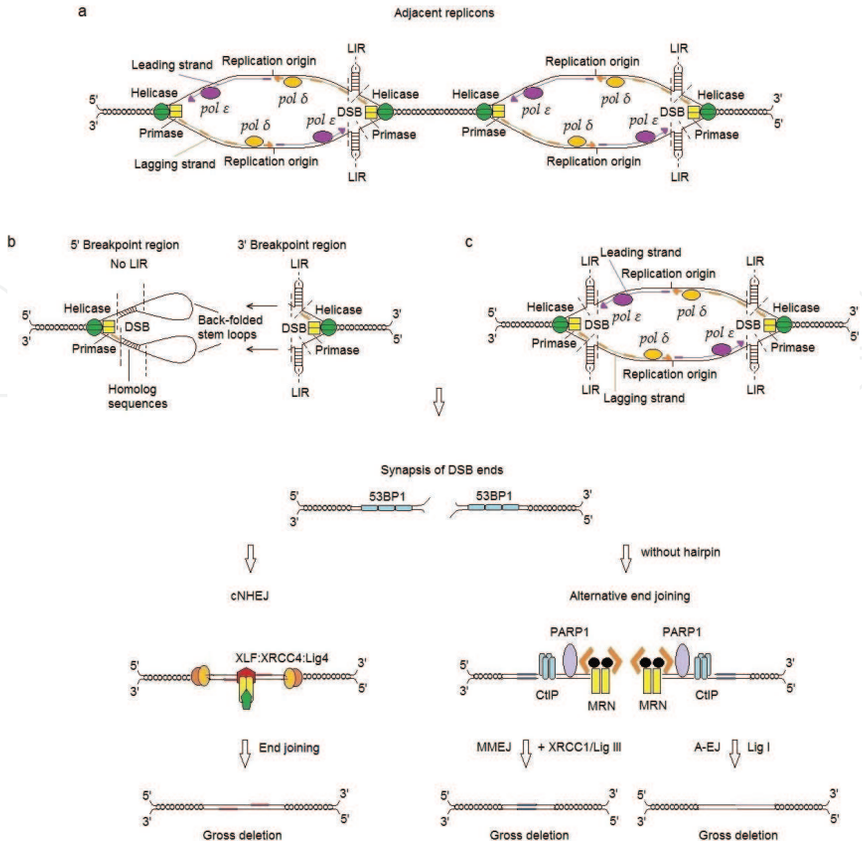

Figure 7. Model mechanisms of long inverted repeat (LIR)-mediated gene deletion during replication (adapted from Ref. [71]). (**a**) LIR-induced gene deletion between adjacent replicons. (**b**) Back-folded stem loop-mediated gene deletion within same replicon. (**c**) 5′ and 3′ LIRs-involved gene deletion within same replicon. Synapsis of DNA double-strand break (DSB) ends is performed by 53BP1 [109]. C-NHEJ can join DNA DSB ends with hairpin or no hairpin via Artemis—DNA-PKcs complex (see **Figure 3**) [74]. In alternative end joining mechanisms, hairpin-opening activity was not yet reported. According to these models proposed here, MMEJ and A-EJ can involve the joining of free DNA ends without hairpin.

interrupted palindrome with 230-bp stem and 20-bp spacer formed a hairpin only on the lagging-strand template in *Escherichia coli*, whereas perfect palindrome generated hairpin on both leading and lagging strand templates during replication [111].

53BP1 can combine free DNA ends between distant sites for repair of double-strand breaks by NHEJ [109]. Through a process dependent on 53BP1 and DNA ligase 4 that are the factors of c-NHEJ, double-strand breaks associated with DNA replication during S phase in BRCA1-deficient cells are aberrantly joined, leading to complex chromosome rearrangements [112]. The ablation of 53BP1 rescues genomic instability in mice expressing BRCA1 lacking N-terminal RING domain [113].

3.1.5. Duplication

Recurrent unbalanced duplications were considerably reported, even though deletions and unbalanced translocations were much more frequent among unbalanced abnormalities in solid tumours [12]. Identical copies of duplicated segments can be distributed as either tandem or interspersed in human genome [10, 114].

Most of germline duplication CNVs (83%) were found to be tandem duplications in direct orientation [115]. A tandem duplication of about 2 Mb at 7q34 produces a novel oncogenic *KIAA1549*:*BRAF* fusion gene capable of transformation in pilocytic astrocytomas [116]. Duplication or gain of chromosome 2p containing the *MYCN* locus by unbalanced translocations is often observed in neuroblastoma cell lines [117].

Segmental duplications (so called low copy repeats, LCRs) in direct or inverted orientation can lead to recurrent chromosomal abnormalities via NAHR mechanism in both germ line and somatic cells, as discussed in Section 3.1.1 (**Figure 1**). A total of approximately 4% of human genome contains segmental duplications, classified as intrachromosomal (2.64%) and interchromosomal (1.44%) duplications [118].

Gene duplication can be produced either by DNA-mediated mechanisms such as unequal crossing over, tandem segmental, chromosomal, and genome duplications or by RNA-based retroposition involving reverse transcription of RNAs from parental genes [119]. In addition, gene duplication can be formed by MMBIR mechanism (**Figure 8**), which is a replication-based mechanism [48].

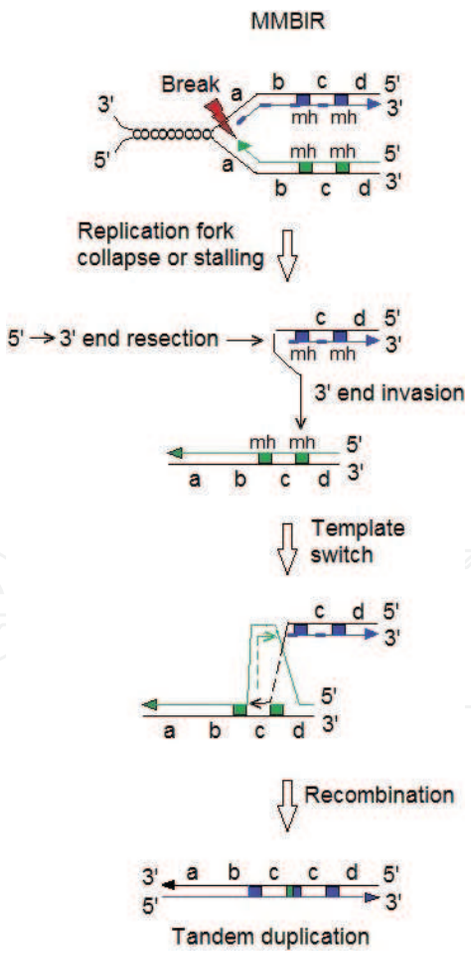

Figure 8. Tandem duplication via microhomology-mediated break-induced replication (MMBIR) mechanism. A single strand break during replication leads to fork collapse or stalling [48]. Then, free 3′ end invades a microhomology (mh) site on the other template, causing template switch. MMBIR results in tandem duplication.

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Tandem duplications are often observed in solid tumours [87–89]. The breakpoints of tandem duplications in solid tumours have mostly no or short microhomology, indicating a template-switching mechanism that does not require microhomology or another non-homology–based mechanism underlying chromosomal duplications [88].

3.1.6. Other chromosomal rearrangements

Normal human genome contains recurrent DNA inversion rearrangements derived from NAHR in particular chromosomes 3, 15, and 19 [57]. The pericentric inv(1) has been more frequently observed in cancer patients (15%), as compared with normal population (4%) [43]. Inversions were much more common (54%) in solid tumours [89].

A small inversion within chromosome 2p generates the *EML4/ALK* fusion gene capable of transformation in non–small-cell lung cancer (NSCLC) cells [120]. Likewise, a pericentric inversion inv(10)(p11.22q11.21) gives rise to *KIF5B/RET* fusion gene that overexpresses chimeric RET receptor tyrosine kinase capable of cellular transformation in NSCLC cells [121].

Inversion is a balanced structural abnormality (**Figure 9a**) and recurs in chromosomes 2, 3, 6, 7, 10, 12, 16, 19 and X in solid tumours [12]. In addition, inversion of chromosome 1 was found in ovary carcinoma, breast carcinoma, seminoma and lymphosarcoma tumours [43].

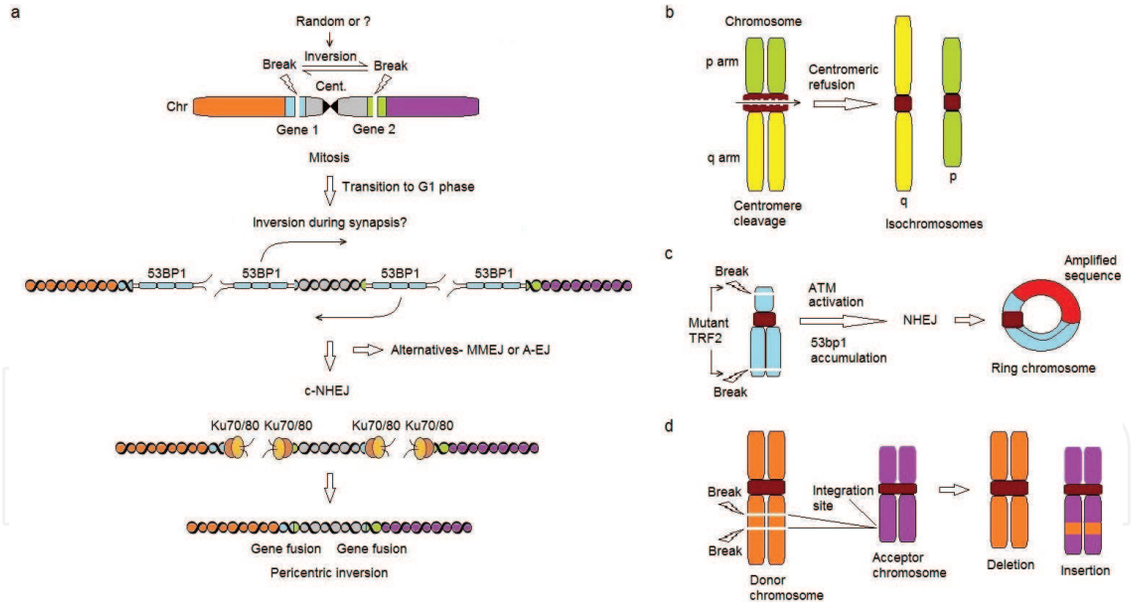

Figure 9. Model mechanisms for the formation of other chromosomal abnormalities observed in solid tumours. (**a**) Model mechanism of a pericentric inversion causing gene fusion is illustrated. NHEJ can result in inversion [49]. According to this model, two chromosomal breaks around centromere in mitosis lead to a pericentric inversion [121]. However, it is not clear how the inversion occurred in which phase of the cell cycle. DSBs occurring in mitosis are not repaired until cells will enter G1 phase [122]. Later, double-strand breaks can be rejoined by NHEJ in G1, see also **Figure 3a** [81], after synapsis of break ends with 53BP1 [109]. (**b**) A model mechanism involving centromere cleavage for isochromosome formation. (**c**) NHEJ-mediated ring chromosome formation. Mutant TRF2 leads to not protect telomeres from DSBs, and 53BP1 promotes NHEJ pathway, which joins the dysfunctional telomeres. (**d**) A chromosomal insertion occurred by three breaks. The fragment resulted from two breaks on the donor chromosome is inserted into integration site at the acceptor chromosome, resulting in an insertion.

An inversion can result from NAHR between inversely oriented LCRs in germ line and somatic cells; see Section 3.1.1 in this chapter (**Figure 1c**). The rearrangement junctions of inversions in breast cancer genomes contain mean 2.5-bp (range, 0–21) microhomology, suggesting that non-homologous end-joining DNA repair involves in formation of inversion [123].

Isochromosomes involving chromosomes 1–17, 21, 22, and X were reported in solid tumours [12]. The i(5p) is a specific chromosome change in bladder cancer, while the i(12p) is seen in almost all tumours of germ cell origin, including seminomas, embryonal cell tumours and teratocarcinomas [124]. Isochromosome 5q along with dmins and hsrs bears extra copies of DHFR gene in the amplified RAD54 deficient cells [125]. In 8% of non-*MYCN* amplified primary tumours, a small number of additional *MYCN* gene copies was shown to be gained through either formation of an isochromosome 2p, or an unbalanced translocation of chromosome 2p including *MYCN* gene, suggesting that isochromosome formation might be one of mediators of gene amplification [126].

For the formation of isochromosomes, multiple mechanisms such as centromeric cleavage, transverse division of the centromere, and NAHR between paralogous LCRs on the sister chromatids were proposed [52, 127, 128]. The centromeric cleavage among these mechanisms was presented (**Figure 9b**).

Constitutional ring chromosomes, 10, 11, 13, 17 and 22, including tumour suppressor gene, were reported in thyroid follicular adenocarcinoma, Wilms tumour, retinoblastoma, neurofibromatosis and meningioma, respectively [129].

Ring chromosomes can form by end-to-end reunion or fusion to other subtelomeric end of the breakage site(s) occurred on either both chromosome arms or one of them, respectively [130], as shown in **Figure 9c**. The ring chromosomes that are observed in atypical lipomatous tumours and other subtypes of mesenchymal neoplasms contain the amplified sequences, primarily from chromosome 12 [131]. In addition, deletion of the shelterin component TRF2 from mouse cells leads to not protect telomeres from DSBs, resulting in activation of ATM kinase and accumulation of 53BP1, promoting the joining of dysfunctional telomeres by NHEJ repair process [132]. NHEJ can generate either a circular chromosome or an unstable dicentric chromosome through a single end joining event between two telomeres [133]. These studies suggest that 53BP1-promoted NHEJ pathway can give rise to formation of a ring chromosome in the absence of TRF2 (**Figure 9c**).

Insertion can be produced by at least three chromosomal breaks, involving a non-reciprocal translocation either between two nonhomologous chromosomes (interchromosomal insertion) or between different regions of same chromosome (intrachromosomal insertion) [134]. During this abnormal process, a chromosomal segment, which is formed by two breaks in a donor chromosome, is inserted into an interstitial region of acceptor chromosome (**Figure 9d**). Large insertions can also be seen in solid tumours [88]. In addition, exonic insertion of L1 and Alu elements was identified in somatic or germline cells in epithelial ovarian cancers [135].

3.1.7. Gene amplification

A large number of oncogenes are amplified in many solid tumours [15, 136]. Amplification of various oncogenes located chromosomes 1–8, 11–14, 16–20 and X, which include AuroraA/AURKA

(bladder, breast and oesophageal), CCND1 (breast, lung, malignant melanoma and oral squamous cell carcinoma), EGFR (colorectal, glioma, lung and oesophageal), ERBB2 (bladder, breast, endometrial, gastric, oesophageal and ovarian), MDM2 (breast, glioma, lung, neuroblastoma and sarcoma), MYC (breast, colorectal, gastric, lung, medulloblastoma and prostate), MYCL1 (lung), *MYCN* (lung, neuroblastoma and rhabdomyosarcoma) and SKP2 (lung, oesophageal and soft tissue sarcoma) was reported in Ref. [15].

The copies of amplified genes are included on either hsrs or dmins [16]. The hsr and dmin are often observed in cell lines derived from solid tumours [137]. Amplicon size of hsr regions varies between 0.8 and 12.7 Mb [138]. The dmins are tiny spherical extrachromosomal structures lacking centromere and telomere, in size of a few Mb [139].

Gene amplification usually results in overexpression of amplified gene, but gene expression level and DNA amplification do not always show an exact match, suggesting that some driver genes can be overexpressed by different mechanisms in the absence of DNA amplification [15, 16]. Up to 44% of highly amplified genes were reported to be overexpressed in breast cancer cell lines, whereas only 10.5% of overexpressed genes demonstrated the increased copy number [140].

Many model mechanisms for the formation of gene amplification have been proposed. First model involves the breakage-fusion-bridge (BFB) cycle, which was first proposed by McClintock [141], between the sister chromatids in mitosis [142]. Lo *et al*. [142] demonstrated that spontaneous telomere loss on a marker chromosome 16 resulted in sister chromatid fusion in a human tumour cell line followed by the amplification of subtelomeric DNA, supporting BFB cycles-mediated gene amplification model (**Figure 10a**).

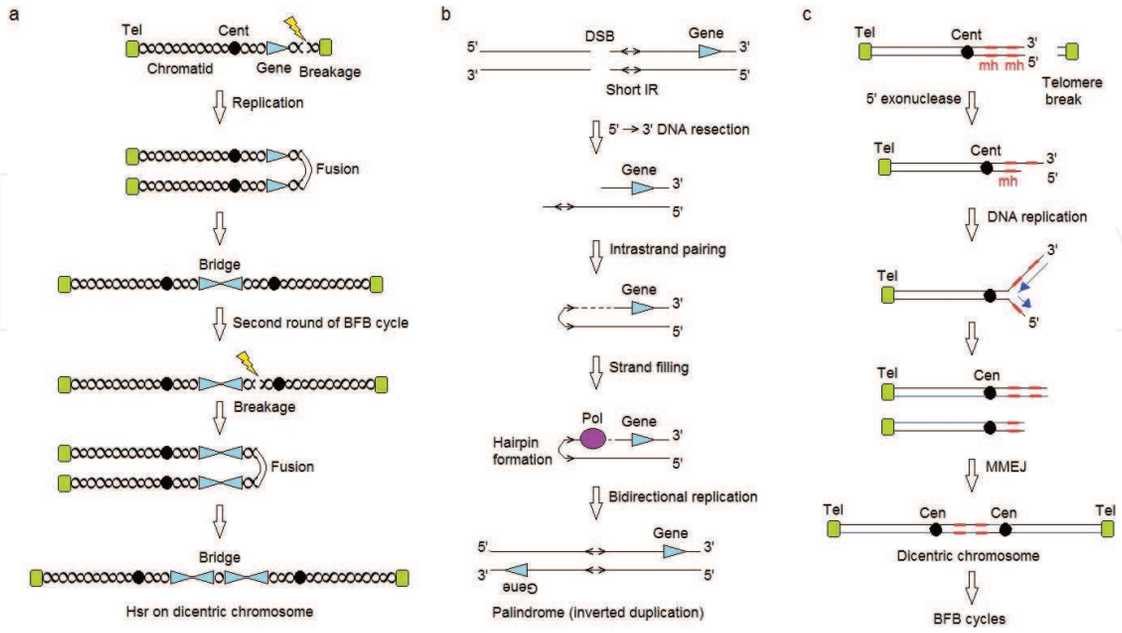

Figure 10. Model mechanisms of gene amplification producing the duplicated units in inverted orientation. (**a**) A gene amplification model involving BFB cycles resulted in hsr on the dicentric chromosome. (**b**) Short IR-mediated amplification model involving intrastrand pairing leading to hairpin formation followed by palindrome. (**c**) Microhomology-mediated gene amplification producing a dicentric chromosome followed by BFB cycles.

Tanaka *et al*. [143] showed that a short inverted repeat, which is introduced into the genome of Chinese hamster ovary cells, promoted the formation of a large DNA palindrome after an adjacent double-strand break. Therefore, the authors proposed an intramolecular recombination model initiating gene amplification through formation of head to head duplication (**Figure 10b**).

Okuno *et al*. [144] have sequenced the junction of head-to-head palindromes of an amplicon containing DHFR amplification in Chinese hamster ovary cells and showed that junction includes a 2-bp microhomology between sites separated by 4 kb. The authors thus proposed a microhomology-mediated recombination model for palindrome formation leading to dicentric chromosome, followed by BFB cycles that trigger the gene amplification (**Figure 10c**).

Difilippantonio *et al*., [145] reported that a recombination activating gene (RAG)-induced DNA cleavage resulted in coamplification of *IgH* and c-*myc* genes after development of lymphoma in NHEJ DNA repair protein Ku and p53 tumour suppressor-deficient mice. The authors proposed a model mechanism involving RAG-induced translocation of *IgH* and c-*myc* in G1, followed by break-induced replication and c-*myc*/*IgH* amplification (head to head) after BFB cycles (**Figure 11**).

On the other side, the replication-based mechanisms for gene amplification were also proposed. Amler and Schwab [26] showed that neuroblastoma cell lines harboured multiple tandem arrays of DNA segments including *MYCN* gene, in head to tail orientation with sizes varying from 100 to 700 kb. The authors proposed that gene amplification may be involved in unscheduled DNA replication, recombination, and dmin formation followed by integration into a chromosome, resulting in subsequent *in situ* multiplication (**Figure 12a**). Schwab [146] also proposed an extra replication model involving excision of amplified segment, integration into a chromosome site, and *in situ* amplification, resulting in hsr (**Figure 12a**).

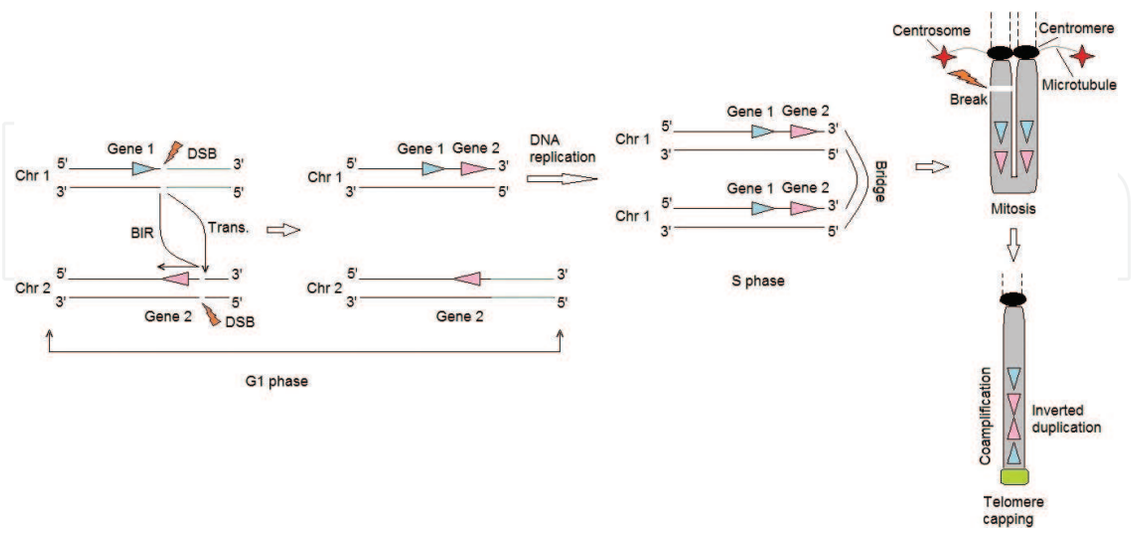

Figure 11. A gene amplification model triggered by double-strand breaks (DSBs). A DSB stimulates break-induced replication (BIR) and unbalanced translocation in G1, resulting in juxtaposition of two different genes. After DNA replication in S phase, BFB cycles cause gene duplication in inverted orientation, resulting in coamplification of two genes.

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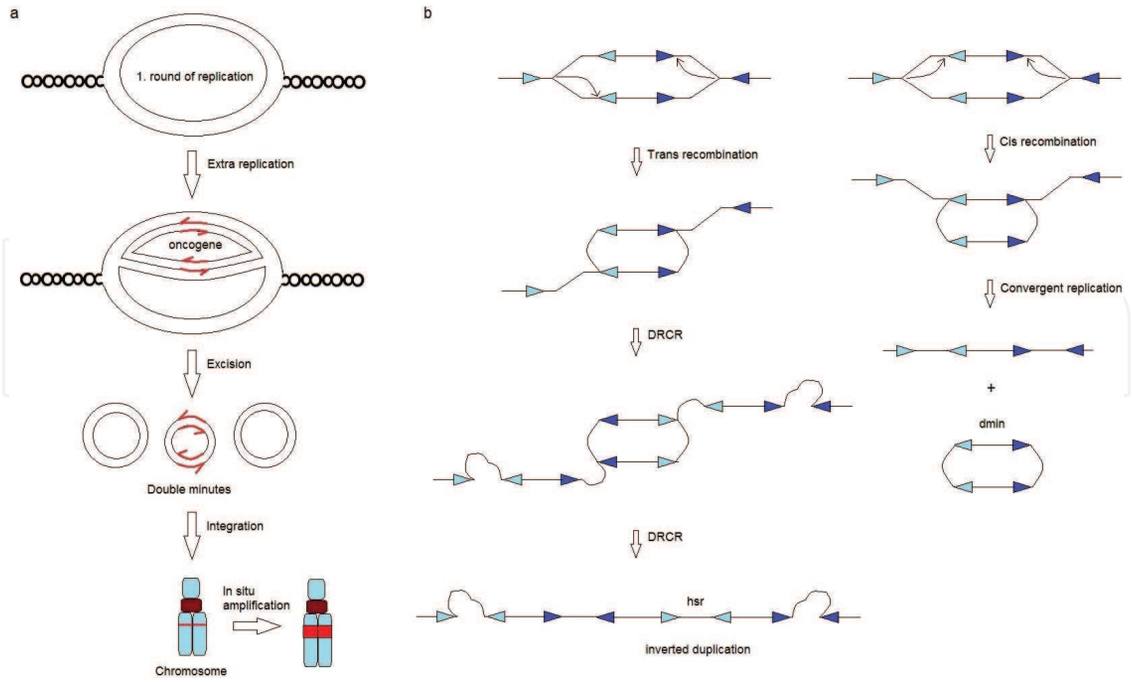

Figure 12. Replication-based mechanisms for gene amplification. (**a**) Extrareplication mechanism involving dmin formation followed by integration of dmin into a chromosome and *in situ* amplification. (**b**) Double rolling-circle replication (DRCR) model by *trans* and *cis* recombination producing hsr and dmin, respectively.

Tower [147] suggested that initial step in amplification of human oncogene and drug-resistant genes may be started by firing of DNA re-replication during S phase. Watanabe *et al*. [148] developed a gene amplification system based on double rolling-circle replication (DRCR) in yeast and mammalian cells, utilising a recombinational process induced by IRs coupled with replication, leading to hsrs and dmins (**Figure 12b**). In addition, Slack *et al*. [149] proposed the FoSTeS mechanism involving long-distance template switching for the gene amplification mechanism (**Figure 4a**).

3.2. Numerical chromosome abnormalities

Changes in chromosome number are frequently observed in particular solid tumours [4]; see **Table 1**. These changes can result from aneuploidy or polyploidy [150]. Aneuploidy refers to abnormal chromosome number deviated from euploidy that is defined as exact multiples of a haploid chromosome set [13, 150]. The chromosome sets in haploid and diploid number are cases of normal euploidy, whereas polyploidy reflects more than two sets of chromosomes, resulting in triploidy (3n), tetraploidy (4n), pentaploidy (5n), and so forth [150].

Aneuploidy involving gain or loss of whole chromosomes, at the same time, can result from some gross chromosomal structural abnormalities including deletion, duplication, unbalanced translocation, and overamplification, as described in other sections of this chapter. This type of aberrant ploidy regarding chromosomal parts is termed segmental or structural aneuploidy [151].

Near-diploid chromosome number (≤68) was predominant (71.8%) in solid tumours compared with near-tetraploid chromosome number (≥69) (19.3%) [13].

Aneuploidy is one of the main implications of chromosomal instability (CIN), leading to tumourigenesis in somatic cells [14]. Errors in cellular processes, such as chromosome condensation, chromatid cohesion, kinetochore assembly, and microtubule/centrosome formation as well as checkpoints, which involved in replication and segregation of chromosomes during mitosis, could lead to the CIN, resulting in chromosomal losses and gains in most cancers (**Figure 13a–d**) [25].

In addition, tetraploid cells can give rise to CIN and aneuploid cell populations *in vivo* (**Figure 13e**) [152]. Increased 4N (G2/tetraploid) fraction along with p53 inactivation during neoplastic progression of Barett's epithelial cells progressed to aneuploidy in Barrett's esophagus, which is a pre-malignant condition [153]. Studies in human cancer cell lines derived from glioblastoma, breast cancer, and melanoma showed direct relation between cell invasiveness and tumour-genome duplication (tetraploid) [154].

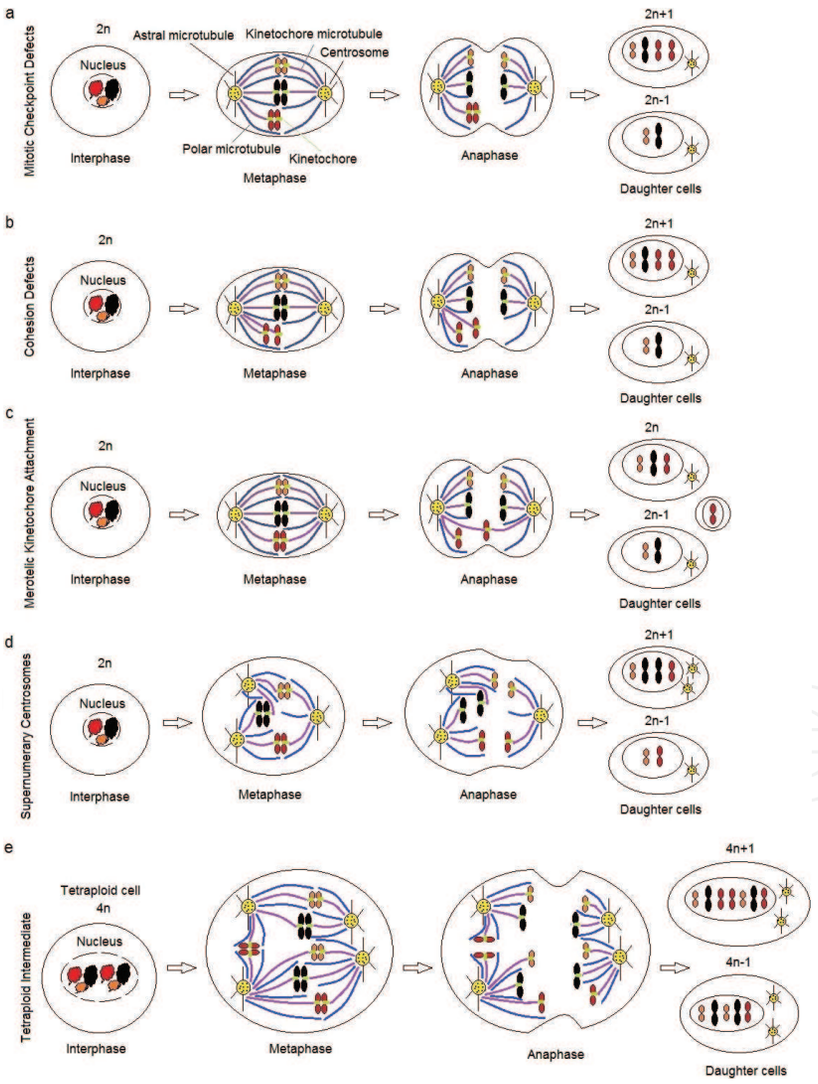

Figure 13. Formation mechanisms of aneuploidy. Errors in (**a**) mitotic checkpoint, (**b**) chromatid cohesion, (**c**) kinetochore attachment, and (**d**) centrosome formation can lead to aneuploidy. (**e**) In addition, tetraploid cells can cause aneuploid cell populations.

In addition, it was proposed that the acquisition of a single trisomy may initiate change from euploidy to aneuploidy as initial event in the development of all malignant solid tumours [155]. Aneuploidy was shown to arise from missegregation of tetraploid nuclei in yeast [156].

In addition, mice with reduced levels of CENP-E motor protein developed aneuploidy and chromosomal instability *in vitro* and *in vivo*, later formed spontaneous lymphomas and lung tumours by an increased rate of aneuploidy in aged animals, suggesting that aneuploidy drives tumourigenesis [157]. Transduction experiments between congenic euploid and trisomic fibroblasts with different oncogenes showed that nearly all aneuploid cell lines divided slowly *in vitro*, relative to matched euploid lines, suggesting that aneuploidy, particularly single-chromosome gains can reveal a tumour suppresive function, but at same time, may facilitate the development of high-complexity karyotypes, leading to advanced malignancies [158].

3.3. MYCN gene amplification in neuroblastoma

Neuroblastoma derived from primitive cells of the sympathetic nervous system is the most common malignancy among childhood cancers [159, 160]. Neuroblastoma is usually a sporadic disease that manifests many complex chromosomal abnormalities such as *MYCN* amplification, 1p deletion, 17q gain, unbalanced t(1;17) translocations, whole chromosome aneuploidies involving trisomies of chromosomes 6, 7, 19 and monosomies of 13, 22, X and Y, as well as LOH of chromosomes 2q, 3p, 4p, 9p, 11q, 14q, 16p and 18q observed in both primary tumours and cell lines [16, 42, 161–163].

MYCN amplification is observed in 18–38% of neuroblastoma cases and multiple neuroblastoma cell lines [45, 164–168]. *MYCN* amplification and 1p36 deletion are important poor prognostic factors in neuroblastoma [17, 164, 169]. We demonstrated that both 1p36 deletion and *MYCN* amplification are significant correlated with undifferentiated tumours [164]. Our group also showed that *MYCN* amplification and 1p36 deletion were associated with high tumour vascularity in neuroblastoma, suggesting close relation of *MYCN* amplification and 1p36 deletion with angiogenesis [170].

The causes and consequences of *MYCN* amplification have been widely studied, but the formation mechanism of *MYCN* amplification still remains to be completely explained. As presented in gene amplifications, some replication-based mechanisms involving the formation of *MYCN* amplification were described. In addition, multiple models of deletion including LIR-mediated gross deletion mechanism were argued in Section 3.1.4.

In this section of chapter, it was investigated the relation between LIRs and *MYCN* amplification. For this aim, LIR distribution in a genomic segment of 16,135,119 bp lying between chromosome 2p25.3 and 2p24.3 loci, including *MYCN* gene locus was first examined. In addition, LIRs were identified in the boundary sequences of amplicons containing *MYCN* gene reported in 14 neuroblastoma cell lines and 42 solid tumours. The results show that a significant association between LIRs and *MYCN* amplification loci. In addition, present data provide some insights into the *MYCN* amplification mechanism.

3.3.1. Material and methods

3.3.1.1. Bioinformatics data

In this study, the boundaries of amplicon units containing *MYCN* gene in 14 neuroblastoma cell lines (CHP134, KP-N-YS, IMR-5, SIMA, NB17, CHP-212, NB7, NB14, NB6, GOTO, NB1, NB5, NB10 and CHP-126) and 42 primary solid tumours (10 lungs, 6 endometriums, 4 bladders, 4 central nervous systems, 3 stomachs, 3 breasts, 2 heads and necks, 2 intestinals, 2 germ cells, 1 ovary, 1 liver, 1 skin, 1 oesaphagus, 1 cervix and 1 adrenal) were analyzed for LIR identification (**Table 2**). Boundary positions and their reference sequences were obtained from COSMIC database that is a catalogue of somatic mutations in cancer [171]. In addition,

Sample	Tumour	B Pos (Mb)	5′ LIR*	<2 kb	In/Out	3′ LIR**	<2 kb	In/Out	Mh
CHP134	NB	15.86–15.95	28	+	O	17	+	O	5
KPNYS	NB	15.47–15.97	6	+	I	18	+	O	5
IMR-5	NB	14.73–15.98	0	−	−	46	+	I	5
SIMA	NB	15.59–15.99	25	+	O	55	+	I	6
NB17	NB	15.61–16.80	8	+	O	1	>	Over	7
CHP212	NB	15.58–16.07	23	+	I	10	+	O	3
NB7	NB	15.66–15.96	3	>	O	12	>	O	5
NB14	NB	15.05–16.94	1	+	I	0	−	−	8
NB6	NB	15.89–15.96	10	+	I	14	+	O	5
GOTO	NB	15.82–15.95	15	+	I	17	+	I	6
NB1	NB	15.90–15.97	23	+	O	18	+	O	3
NB5	NB	15.16–16.26	0	−	−	0	−	−	2
NB10	NB	15.69–15.97	3	+	O	18	+	O	4
CHP126	NB	15.41–15.99	1	>	Over	46	+	O	2
1	EC	15.52–16.05	5	>	I	15	+	Over	2
2	BC	5.26–17.60	0	−	−	0	−	−	4

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Sample	Tumour	B Pos (Mb)	5′ LIR*	<2 kb	In/Out	3′ LIR**	<2 kb	In/Out	Mh
3	LSCC	0.013–69.96	8	+	I	13	+	I	14
4	EC	7.06–17.55	1	>	Over	0	–	–	12
5	GLI	15.15–15.95	3	+	O	17	+	I	6
6	LAC	12.47–16.93	15	+	I	0	–	–	2
7	HNSCS	15.14–22.35	3	+	I	4	+	I	13
8	EC	15.72–16.05	2	>	I	10	+	I	5
9	CAC	15.93–16.09	44	+	O	37	+	I	3
10	OSC	6.04–20.35	0	–	–	26	+	O	6
11	BC	12.20–18.09	3	+	I	0	–	–	8
12	HCC	14.96–19.29	1	+	I	5	+	I	5
13	GLI	14.57–16.90	3	+	I	4	+	Over	8
14	LSCC	13.26–16.35	2	+	O	18	+	O	6
15	GLI	15.75–16.11	0	–	–	9	+	I	8
16	LAC	15.65–16.00	15	+	Over	21	+	I	4
17	MM	0.013–16.33	8	+	I	1	>	I	7
18	OC	15.92–16.98	35	+	I	10	+	I	2
19	SAC	13.84–17.27	2	+	I	0	–	–	7
20	CSCC	10.61–31.02	27	+	I	3	+	I	2
21	GLI	12.83–16.77	5	+	O	4	+	I	10
22	LAC	7.60–16.41	67	+	O	1	>	O	5
23	GCT	0.38–37.53	2	+	I	4	+	O	3
24	EC	0.013–19.41	8	+	I	7	+	Over	9
25	CAC	0.013–32.27	8	+	I	86	+	I	6

Sample	Tumour	B Pos (Mb)	5′ LIR*	<2 kb	In/Out	3′ LIR**	<2 kb	In/Out	Mh
26	BRC	13.15–15.95	0	−	−	17	+	I	5
27	BC	13.02–17.49	27	+	I	0	−	−	7
28	LAC	15.88–17.36	8	+	O	3	>	Over	5
29	HNSCC	4.03–64.05	1	>	O	35	+	I	4
30	LAC	0.013–25.64	8	+	I	19	+	O	2
31	LSCC	14.44–17.81	0	−	−	8	+	I	5
32	LSCC	15.84–16.98	10	+	I	10	+	I	4
33	BRC	5.56–16.48	3	>	Over	1	>	O	6
34	SAC	1.77–29.73	5	+	I	0	−	−	2
35	EC	15.69–15.98	7	+	Over	52	+	I	2
36	EC	15.61–17.52	9	+	O	1	+	I	4
37	BRC	0.013–31.19	8	+	I	1	>	I	5
38	SAC	14.93–16.74	2	+	I	1	+	I	3
39	BC	11.94–20.22	6	+	I	5	+	O	2
40	ACC	15.59–15.95	26	+	I	17	+	I	5
41	GCT	10.81–22.55	28	+	O	2	+	I	4
42	LAC	14.72–15.97	1	+	O	18	+	O	2
T: 56			T: 562	42 < 2 kb 75%	I: 28 O: 16	T: 757	40 < 2 kb 71.43%	I: 26 O: 16	M:5.18 (2–14)

[a]All amplicons including *MYCN* gene analyzed here are located at the short arm (p) of chromosome 2.

*$P < 0.05$, compared with control group.

**$P < 0.01$, compared with control group.

Abbreviations: ACC, adrenal cortical carcinoma; BC, bladder carcinoma; BRC, breast carcinoma; CAC, colon adenocarcinoma; CSCC, cervix squamous cell carcinoma; EC, endometrioid carcinoma; GCT, germ cell tumour; GLI, glioma; HNSCS, head and neck squamous cell carcinoma; LAC, lung adenocarcinoma; LSCC, lung squamous cell carcinoma; MM, malignant melanoma; NB, neuroblastoma; OC, oesophagus carcinoma; OSC, ovary serous carcinoma; SAC, stomach adenocarcinoma. B Pos, boundary position; In/Out, inside/outside the amplicon; LIR, long inverted repeat; M, mean; Mh, microhomology; Over, on the boundary of amplicon; T, total.

Table 2. Boundary positions, microhomology and LIR analyses at the amplicon units containing *MYCN* gene locus in neuroblastoma cell lines and other solid tumours[a].

contiq sequences (NCBI acc no: NT_005334.17) of *Homo sapiens* chromosome 2p containing *MYCN gene* for examining the LIR distribution were downloaded from NCBI website [172].

In addition, microhomology analysis between 150-bp DNA sequences spanning 5′ and 3′ boundaries was performed using Dialign software program [173].

3.3.1.2. LIR identification

LIRs with stem length ≥20 bp, stem identity ≥70%, and internal spacer (loop length) of 0–10 kb were identified at ±10 kb (a total of 20 kb) segments encompassing the rearrangement (boundary) sites of the amplicon units including *MYCN* gene, using the inverted repeat finder (IRF) software [174] in cell lines and primary tumours, as described in Ref. [71]. In addition, LIR distribution was determined in a genomic segment of 16,135,119 bp lying between chromosome 2p25.3 and 2p24.3 loci, including *MYCN* gene locus. LIRs with same features were also investigated at the DNA segments of 20 kb in control group (n = 61), including the randomly selected genes that were not shown to associate with any DNA amplification or deletion in literature and HGMD site, respectively [15, 136, 175]. Total LIR numbers of both amplification boundaries and control gene segments were determined (**Table 2**) and statistically compared with each other.

3.3.1.3. Statistical analysis

Mann-Whitney U test was used for statistical comparison of mean ranks of LIR numbers between test and control groups. Two-sided *P* values <0.05 were considered statistically significant. Analyses were performed using SPSS 11.0 software (Chicago, USA).

3.3.2. Results and discussion

We previously showed that Kelly neuroblastoma cell line harbours only one of chromosomes 2 in 23 metaphases using FISH method (Aygun N and Altungoz O, unpublished data). We also confirmed that *MYCN* locus is deleted on this unique chromosome 2, and hsrs containing *MYCN* amplification are located only two chromosomes 17 (**Figure 14**). In addition, I revealed a significant association between LIRs and breakpoint regions of gross deletions in human cancers and inherited diseases [71]. To investigate the relation between LIRs and mechanism of the *MYCN* gene amplification in this chapter, I examined the distribution of LIRs on a genomic segment of 16,135,119 bp lying between p25.3 and p24.3 loci of the short arm (p) of chromosome 2, which contains the *MYCN* gene (**Figure 15a**).

A total of 6839 LIRs with stem length ≥20 bp, stem identity ≥70% and internal spacer (loop length) of 0–10 kb were identified in this genomic segment (NCBI acc no: NT_005334.17), using IRF software (**Figure 15b**). Of these identified LIRs, 5155 (75.38%) are distributed along second half (9–17 Mb) of this segment (**Figure 15b**), containing *MYCN* locus at 2p24.3 (**Figure 15a**). Of second half LIRs, a total of 1751 (33.97%) have stem length ≥20 bp, stem identity ≥70% and loop length of 0–2 kb (**Figure 15c**). Of this second group LIRs, a total of 330 (18.85%) have stem length ≥20 bp, stem identity ≥85%, and loop length of 0–2 kb (**Figure 15d**), which may be potentially recombinogenic [177]. Second half of chromosome 2p25.3–2p24.3

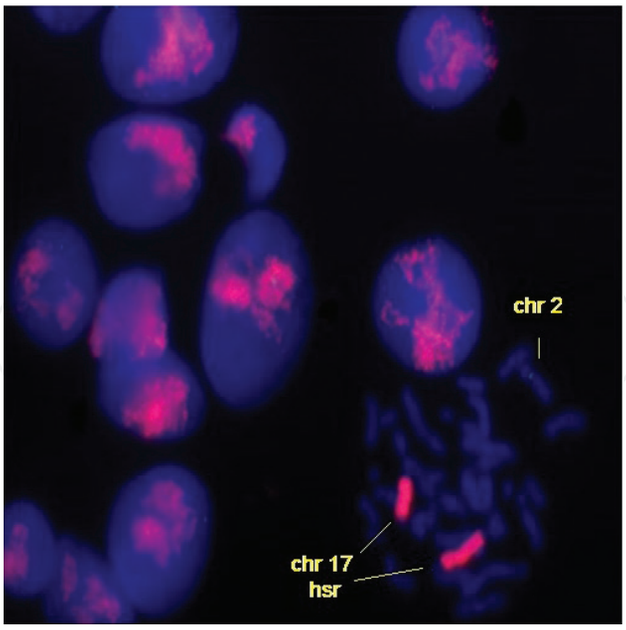

Figure 14. A metaphase demonstrating deleted 2p24 locus on single chromosome 2 and two hsrs including *MYCN* gene on chromosomes 17 in Kelly neuroblastoma cell line. Fluorescence *in situ* hybridization (FISH) probe: *MYCN* gene (2p24)/Chromosome 2 Alpha-Satellite (red/green, Qbiogene, cat. no., PONC0224).

also includes an SRO of ~68 kb (**Table 2**: 15,900,307–15,968,674, **Figure 15b**) between amplicon units analyzed here, all of them contain *MYCN* gene (NT_005334.17, 15,930,557–15,930,962).

It was found that two common fragile sites (cFS) spanning 747-kb *FRA2Ctel* and 746-kb *FRA2Ccen* at 2p24.3 and 2p24.2, respectively, are separated by a 2.8-Mb non-fragile region containing *MYCN* [178]. The authors also determined that 56.5% of *MYCN* amplicons from neuroblastoma cell lines and primary tumours are clustered in *FRA2C*, suggesting that *MYCN* amplicons could be formed due to extrareplication rounds of unbroken DNA secondary structures that accumulate at *FRA2C*.

To investigate significance of the association between LIRs and *MYCN* gene amplification, LIRs were identified at the ±10 kb segments encompassing both 5′ and 3′ rearrangement boundaries of amplicon units including *MYCN* gene, using IRF software in neuroblastoma cell lines and primary solid tumours. LIRs were also investigated at 20-kb segments of the genes in control group. In conclusion, statistical analysis showed that mean LIR number was significantly higher in both 5′ and 3′ rearrangement boundaries of the amplicon units including *MYCN* gene than in control group, respectively ($P < 0.025$; $P < 0.004$; **Table 2**). Of 5′ boundaries in 56 amplicon units, 49 (87.5%) have at least one LIR with stem length ≥20 bp, stem identity ≥70%, and loop length of 0–10 kb, while 47 (83.93%) of 3′ boundaries include at least one LIR with same features (**Table 2**). Of these 49 5′ LIRs and 47 3′ LIRs, 28 (57.14%) and 26 (55.32%) are inside the amplicon unit, respectively. In addition, 42 (85.71%) of 49 5′ LIRs have loop length <2 kb, while 40 (85.11%) of 47 3′ LIRs contain the loops <2 kb (**Table 2**). Of these 42 5′ and 40 3′ LIRs with loops <2 kb, 26 (61.9%) and 24 (60%) were found inside the amplicon

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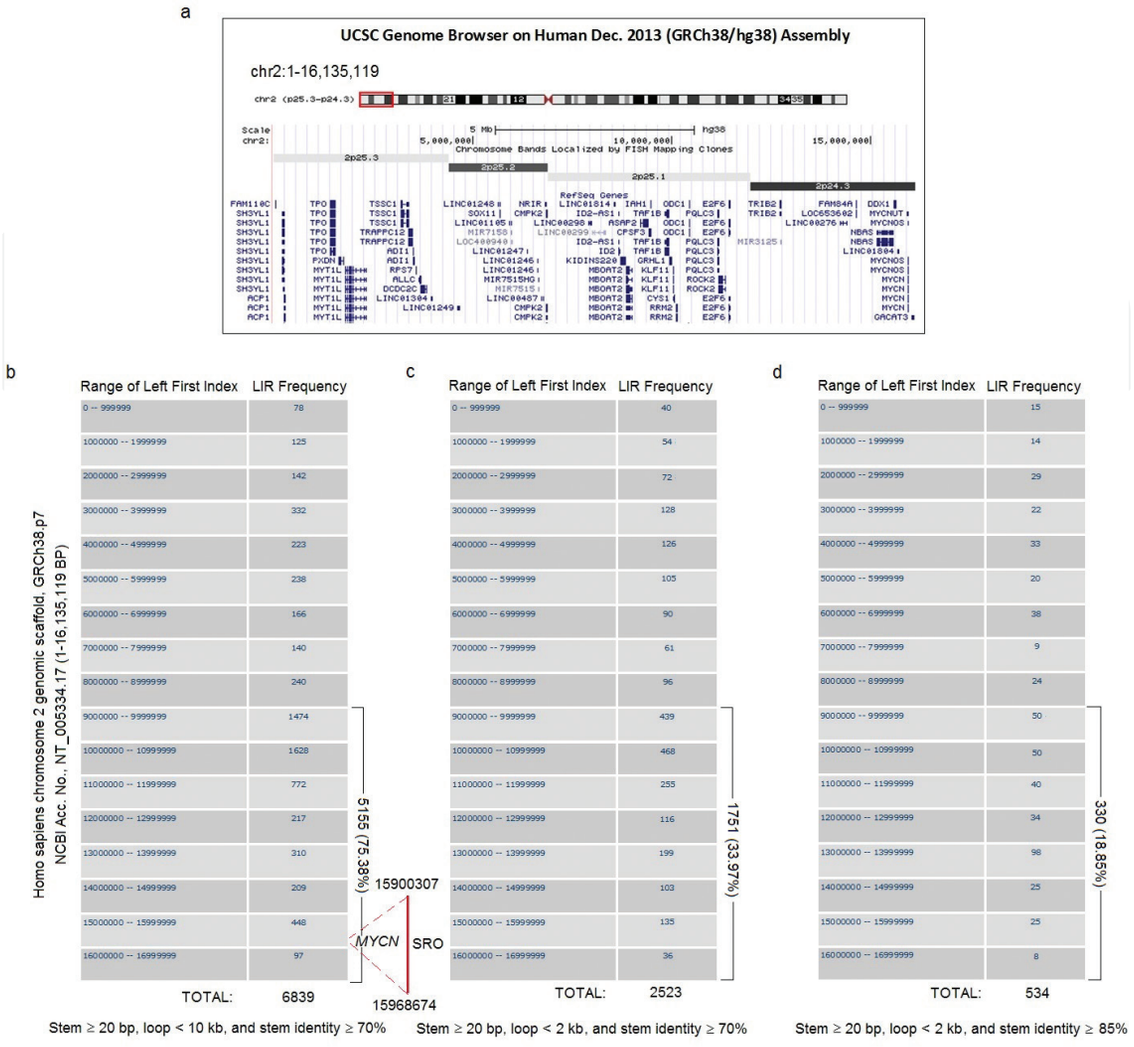

Figure 15. LIR identification in chromosome 2p containing *MYCN* gene. (**a**) LIR distribution was examined in a genomic segment (NT_005334.17) of 16,135,119 bp lying between chromosome 2p25.3-p24.3. Map of this chromosomal region was obtained from UCSC genome browser [176]. (**b**) LIR frequency with stem length ≥20 bp, stem identity ≥70%, and loop length <10 kb. (**c**) LIR frequency with stem length ≥20 bp, stem identity ≥70%, and loop length <2 kb. (**d**) LIR frequency with stem length ≥20 bp, stem identity ≥85%, and loop length <2 kb.

unit, respectively, suggesting that LIRs inside the amplicon unit could potentially generate a hairpin at single-stranded DNA and break the DNA strand during replication. Hairpin structure was shown to form at an interrupted LIR with 111-bp stem and 24-bp internal spacer on both leading and lagging strand templates during replication [110]. In addition, a single-strand DNA break may cause replication fork stalling or collapse [48]. However, LIRs outside a replicon, near the rearrangement boundaries, may also cause a replication fork stalling through formation of cruciform extrusion [110].

Of 49 5′ LIRs, 28 (57.14%) have stem length ≥20 bp, stem identity ≥75% (18 LIRs with stem identity ≥85% were found, 14 of them were inside the amplicon unit) and loop length of 0–2 kb,

while 37 (78.72%) of 47 3′ LIRs have stem identity ≥75% (9 LIRs with stem identity ≥85% were found, six of them were inside the amplicon unit; data not shown in **Table 2**). LIRs with stem identity >85% were highly recombinogenic in human and other organisms [177]. In addition, the long Alu IRs with 75% stem identity can cause a mild replication blockage in *E. coli* [106]. Therefore, present data suggest that LIRs with 85 and 75% stem identities identified here in the boundaries of the amplicons including *MYCN* gene can be potentially recombinogenic or can lead to at least mild replication blockage, respectively.

Present results also showed that a microhomology of mean 5.18 bp ranging from 2 to 14 bp between sequences of 5′ and 3′ boundaries in the amplicons (**Table 2**). Microhomology between 0 and 15 bp can be a signature for NHEJ, MMEJ, MMBIR, or FoSTeS mechanisms [86, 149, 179].

On the other side, a recombination hotspot harbouring tandem amplicons in head to tail orientation at 17q21 that is not linked to common fragile sites, containing *ERBB2* gene locus, was discovered, indicating an alternative mechanism other than BFB model in oncogene amplification [180]. Interestingly, additional copies of *MYCN* oncogene in Kelly cell line are also integrated at 17q21 locus, whereas it is deleted at original 2p24 locus (**Figure 14**). In addition, it was shown that multiple tandem arrays of DNA segments including *MYCN* gene were in head to tail orientation with sizes varying from 100 to 700 kb in neuroblastoma cell lines [26]. In this chapter, the boundaries of the amplicons containing *MYCN* gene located 2p were analyzed for LIRs, however, LIRs at 17q21 locus remained investigated.

Taken together, present results suggest that LIRs could be contributed to induce *MYCN* amplification possibly through either replication fork stalling or break-induced replication dependent on microhomology during replication in chromosome 2p (**Figure 16**). In addition, LIRs may cause deletion of *MYCN* gene at 2p24 and trigger its insertion into chromosome 17q21 involving nonhomologous recombination in Kelly cell line. After insertion, 2p24 hsr including *MYCN* gene at 17q21 might be arisen from again replication fork stalling or break-induced replication dependent on microhomology.

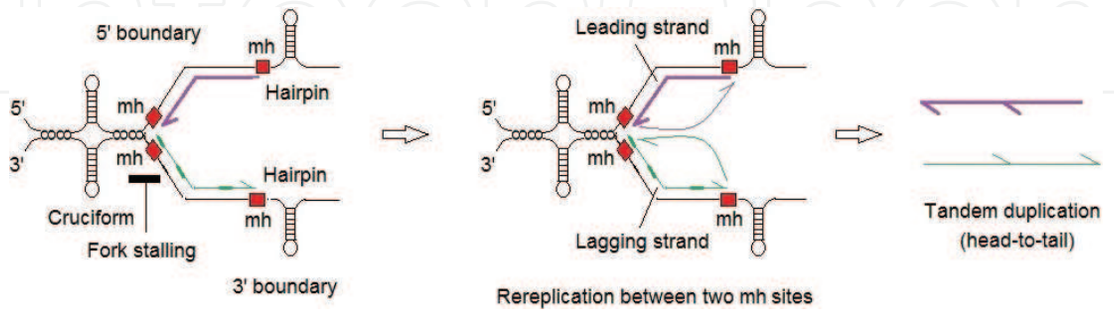

Figure 16. A model of long inverted repeat (LIR)-induced gene amplification. A cruciform near 5′ boundary of an amplicon unit can cause the fork stalling during replication. Hairpins formed at both leading and lagging strand templates may slow the DNA synthesis. Both cruciform and hairpin structures could trigger a rereplication between two microhomology (mh) sites located 5′ and 3′ boundaries of the amplicon, leading to the formation of head-to-tail tandem duplication.

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I thank "Nature Publishing Group (NPG)" for reusing to be adapted without obtaining permission in this chapter (**Figure 7a–c**) of **Figures 6** and **7** in my article entitled "Correlations between long inverted repeat (LIR) features, deletion size and distance from breakpoint in human gross gene deletions" [71], published in Scientific Reports. NPG provides the right to reuse the figures without obtaining permission for the authors of previously published articles, which are licenced under a Creative Commons Attribution 4.0 International Licence (http://creativecommons.org/licenses/by/4.0).

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