Original Research

Whole-chromosome arm acquired uniparental disomy in cancer development is a consequence of isochromosome formation

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Abstract

Using SNP-based microarray data from The Cancer Genome Atlas (TCGA), we investigated isochromosomes (deletion of one arm and duplication of the other arm) and related acquired uniparental disomy in 12 tumor types. We observed a high frequency of isochromosomes (25.98%) across all type of tumors except thyroid cancers. The highest frequency of isochromosomes was found in lung squamous cell carcinoma (54.18%). Moreover, whole-chromosome arm acquired uniparental disomy (aUPD) was common in the deleted arms of isochromosomes. These data are consistent with whole-chromosome arm aUPD likely being a consequence of isochromosomes formation. Our findings implicated aUPD as occurring through error-prone DNA repair of a deleted arm or segment of a chromosome that leads to homozygosity for existing alterations. Isochromosomes were significantly more frequent in *TP53* mutated samples than wild types in 6 types of tumors with loss of *TP53* function potentially contributing to development of isochromosomes. Isochromosomes are common alterations in cancer, and losing one arm of a chromosome could result in duplication of the lost arm. Duplication of the remaining arm leads promulgation of the effects on any defects in the remaining allele, due to subsequent homozygosity.

Neoplasia (2022) 25, 9–17

Introduction

DNA damaging agents or defects in DNA replication can cause DNA double-strand breaks (DSBs). DSBs in normal cellular conditions are efficiently repaired through two main mechanisms: homologous recombination (HR), a high fidelity approach, and non-homologous end joining (NHEJ) an error prone approach. HR uses the intact DNA strand as a template, allowing accurate repair of DNA damage. NHEJ dominates during the G1 to early S phase of the cell cycle whereas HR is mainly used in the late S and G2 phases [1]. Incorrectly repaired DSBs can promote chromosomal aberrations including structural and copy number alterations [2]. Aberrant DNA damage repair can result in isochromosomes, which are abnormal chromosomes with one chromosome having identical arms due to loss of one arm, and duplication of the remaining arm. Thus isochromosomes have 3 copies of one arm, and one copy in the other arm. Isochromosomes can develop in mitosis or in meiosis through multiple mechanisms. Isochromosomes have been reported mostly in rare syndromes [3–6] and hematologic malignancies [7–10]. i17q is the most commonly reported isochromosome in hematologic malignancies [7–11]. However, characterization of isochromosomes in solid tumors has been mostly evaluated in cell lines. Here we studied the frequency and distribution of isochromosomes across the original 12 solid tumor lineages in The Cancer Genome Atlas (TCGA). In addition to isochromosomes, we observed whole-chromosomal arm and segmental (telomeric and interstitial) acquired uniparental disomy (aUPD) in the replaced arm of isochromosomes. aUPD, as first described by Engel [12], can occur segmentally or involve whole- chromosomes. Segmental

aUPD has been proposed to occur through mitotic recombination [13,14], while in whole-chromosome aUPD, one chromosome is lost and the remaining chromosome is duplicated [15]. Bridge-breakage-fusion has also been proposed to underlie UPD [16]. However, mechanisms leading to whole-chromosome arm aUPD have remained unclear. Here, we propose that following loss of a whole-chromosome arm, a partial or whole-chromosome arm is duplicated using the homologous chromosome as a template with consequent segmental or whole-chromosome arm aUPD.

Materials and methods

Samples

SNP-based microarray and mutation data were retrieved from The Cancer Genome Atlas (TCGA) (https://portal.gdc.cancer.gov) and XENA (https://xenabrowser.net). A total of 9340 (4,670 tumor and 4670 matching normal) samples were analyzed. In this study we included the 12 sites first provided to TCGA that include high-grade serous ovarian cancer (HGSOV, 539 samples), cervical squamous cell carcinoma (CESC, 289 samples), uterine corpus endometrial carcinoma (UCEC, 509 samples), esophageal carcinoma (ESCA, 184 samples), stomach (gastric) adenocarcinoma (STAD, 421 samples), colon adenocarcinoma (COAD, 437 samples), rectum adenocarcinoma (READ, 148 samples), lung squamous cell carcinoma (LUSC, 419 samples), lung adenocarcinoma (LUAD, 473 samples), head and neck squamous cell carcinoma (HNSCC, 448 samples), skin cutaneous melanoma (SKMC, 376 samples), and thyroid cancer (THCA, 467 samples). We also analyzed Affymetrix SNP6.0 genotyping data from SW837 colorectal adenocarcinoma cell lines (GSM888771) [17] and NCI H209 lung cancer cell line (GSM888482) for which karyotypes are well known [18].

Microarray and statistical analyzes

Genome-wide isochromosome (excluding acrosentric chromsomes), acquired uniparental disomy and deletion analyzes were identified by using CNAG v4.0 (http://www.genome.umin.jp). Array based data provides both information about alleles and DNA intensity for each position. Isochromosomes are defined when breakpoints are located at the centromeric (p10-q10) regions and results with deletion of the whole-arm of chromosome and duplication of the other whole-chromosome arm. Thus, monosomy in one chromosomal arm and trisomy in the other chromosomal arm is observed in cells that harbor isochromosomes. Whole-chromosome arm deletion is defined as loss of a chromosomal arm from the telomere to centromere. We use 'simple whole-chromosome arm deletion' to describe a chromosome arm deletion to avoid confusion and separate this from generation of isochromosomes. Simple whole-chromosome arm deletion results in monosomy in the deleted arm without trisomy in the other chromosome arm. Whole-chromosome arm aUPD is defined if aUPD is observed in the deleted arm (from telomere to centromere) of isochromosomes or in a simple whole-chromosome arm deletion. Segmental aUPD is defined where aUPD is observed in part of deleted arm of isochromosomes or in simple deleted whole-chromosome arm. A two-tailed student-t test was used to identify the difference of frequency of whole-chromosome arm aUPD between isochromosome and simple whole-chromosome arm deletion, and frequency of isochromosomes between samples with and without mutations (for the most common 14 mutations, and cohesion complex genes). We also performed Pearson chi-squared analysis to test the association between mutations and samples with isochromosomes. We applied the Benjamini-Hochberg false discovery rate to evaluate the p values [19]. Statistical analysis was performed in STATA v10 (STATA Corp., College Station, TX).
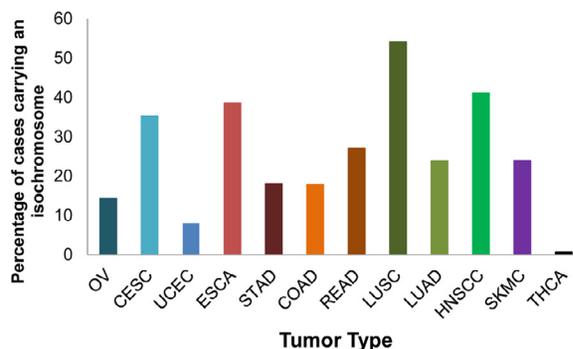
Figure 1. Frequency of isochromosomes across 12 tumor types. Frequency of isochromosomes in HGSOV, CESC, UCEC, ESCA, STAD, COAD, READ, LUSC, LUAD, HNSCC, SKMC, and THCA. Percentage was calculated based on all samples.

Results

Frequency and distribution of isochromosomes

We used TCGA-generated SNP-based Affymetrix microarray data to identify isochromosomes for 12 tumor types. We found that 25.98% samples carried at least one isochromosome. In total, we identified 1405 isochromosomes across the 4,670 tumor samples we analyzed. We found the highest frequency of isochromosomes in lung squamous cell carcinoma (54.18%), followed by HNSCC (41.07%), esophageal cancer (38.59%), cervical cancer (35.29%), rectal cancer (27.03%), melanoma (23.94%), lung adenocarcinoma (23.89%), gastric cancer (18.05%), colon cancer (17.88%), ovarian cancer (14.29%), and uterine cancer (8.06%), with the lowest prevalence of isochromosomes being identified in thyroid cancer (0.64%) (Figure 1). Thyroid cancers were excluded from further analysis due to low numbers of isochromosomes.

The most common isochromosomes across cancers were i3q, i5p, i6p, i8q and i20q, however the frequencies varied among the cancer types (Figure 2). Isochromosomes 5p (i5p) and i8q were the most frequent in ovarian cancer, i3q and i5p in cervical cancer, i3q and i8q in uterine cancer, i3q and i5p in esophageal cancer, i5p and i8q in gastric cancer, i8q and i20q in colon and rectal cancer, i3q and i5p in lung squamous cell carcinoma, i5p and i8q in lung adenocarcinoma, i3q and i8q in head and neck squamous cell carcinoma, and i6p and i8q in melanoma. Isochromosome at 8q was the most common recurrent isochromosome in ovarian, uterine, gastric, colon, rectal, lung adenocarcinomas, head and neck cancers and melanoma. i5p was frequent in ovarian, cervical, esophageal, lung squamous cell carcinoma, lung adenocarcinoma, and gastric cancers, while i3q was prevalent in head and neck squamous cell cancers, cervical, esophageal, uterine and lung squamous cell cancers. The distribution of the most common isochromosomes is shown in Figure 2. In addition, we observed infrequent isochromosomes across the 12 tumor types as shown in Table 1. Based on analysis of the most common isochromosomes across 11 tumor types, the frequency of whole-chromosome arm deletion due to isochromosomes is 49% and due to simple whole-chromosome arm deletion is 51%. Although chromosome 3p arm deletion has been observed to be a frequent alteration in head and neck squamous cell carcinomas [20], we found that a significant portion of whole-chromosome arm deletion at 3p is due to formation of i3q (35.14%; 78/222), and simple chromosome deletions explained the majority (64.86%; 144/222) in HNSCCs (Supplemental Table S1). To validate the analytical approach we used, based on analyzing array data we analyzed array-based genotyping data from SW837 colorectal adenocarcinoma cell line and found isochromosome in 20q (Figure 3), which was previously shown in karyotyping analysis [17].
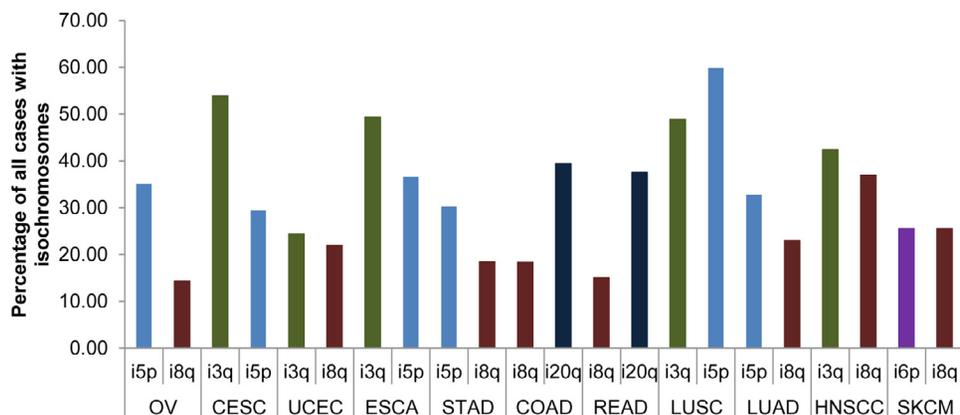
Figure 2. Frequency of the most common isochromosomes in 11 tumor types. The most recurrent isochromosomes and their frequency in each tumor type are different. The most frequent observed isochromosomes are i5p and i8q in OV, i3q and i5p in CESC, i3q and i8q in UCEC, i3q and i5p in ESCA, i5p and i8q in STAD, i8q and i20q in COAD, i8q and i20q in READ, i3q and i5p in LUSC, i5p and i8q in LUAD, i3q and i8q in HNSCC, and i6p and i8q in SKMC. Percentage was calculated based on samples with isochromosomes.

Table 1

Isochromosomes across 12 tumor types.

Tumor type	Isochromosomes (frequency of isochromosomes)
HGSOV	1q (6.49%), 2p (1.30%), 3q (11.69%), **5p (35.06%)** 6p (9.09%), 7p (1.30%), 7q (6.49%), **8q (14.29%),** 9p (2.60%), 10p (6.49%), 11q (2.60%), 12p (11.69%),18p (5.19%), 20q (3.90%), Xq (1.30%)
CESC	1q (5.88%), 2p (1.96%), **3q (53.92%), 5p (29.41%),** 6p (1.96%), 7p (1.96%), 7q (0.98%), 8q (1.96%), 9q (1.96%), 10p (1.30%), 11p (1.30%), 12q (1.96%), 16p (1.96%), 17q (4.90%), 18p (2.94%), 19q (6.86%), 20q (3.92%), Xp (1.96%), Xq (1.96%)
UCEC	1q (4.88%), 2p (2.44%), **3q (24.39%)**, 5p (9.76%), 7p (2.44%), 7q (4.88%), **8q (21.95%)**, 9p (7.32%), 10p (2.44%), 12p (2.44%), 16p (7.32%), 17q (2.44%), 18p (9.76%), 20q (4.88%)
ESCA	1p (1.41%), 1q (7.04%), **3q (49.30%), 5p (36.62%),** 7p (5.63%), 7q (1.41%), 8q (7.04%), 9q (4.23%), 10p (2.82%), 12p (1.41%), 12q (1.41%), 17q (7.04%), 18p (5.63%), 19q (2.82%), 20q (7.04%)
STAD	1q (10.53%), 3q (3.95%), **5p (30.26%),** 7p (7.89%), **8q (18.42%),** 9q (1.32%), 10p (6.58%), 10q (1.32%), 11q (2.63%), 12q (1.32%), 16p (3.95%), 17q (3.95%), 18q (1.32%), 19q (13.16%), 20q (14.47%)
COAD	1q (12.68%), 3q (2.82%), 4q (1.41%), 5p (8.45%), 7p (2.82%), **8q (18.31%)**, 9p (2.82%), 10p (4.23%), 11p (1.41%), 12p (1.41%), 16p (1.41%), 16q (1.41%), 17q (11.27%), 18p (1.41%), **20q (39.44%)**
READ	1q (7.32%), 4q (4.88%), 5p (7.32%), 6p (2.44%), 7p (2.44%), **8q (14.63%)**, 9p (2.44%), 11p (4.88%), 12p (2.44%), 16q (2.44%), 17q (12.20%), 18p (7.32%), **20q (36.59%)**
LUSC	1q (8.81%), 2p (0.44%), **3q (48.90%)**, 4q (0.44%), **5p (59.91%)**, 6p (0.88%), 6q (0.44%), 7q (0.44%), 8q (4.85%), 9p (1.32%), 9q (0.88%), 10p (0.88%), 12p (2.20%), 16q (0.88%), 17q (3.08%), 18p (1.32%), 19q (1.76%), 20p (0.44%), 20q (5.29%)
LUAD	1q (12.39%), 3q (7.08%), 4p (1.77%), **5p (32.74%)**, 6p (9.73%), 7p (6.19%), **8q (23.01%)**, 10p (0.88%), 12p (0.88%), 16p (7.08%), 17q (9.73%), 18p (1.77%), 18q (0.88%), 20q (1.77%), Xq (0.88%)
HNSCC	1q (2.72%), 2p (0.54%), **3q (42.39%)**, 4p (0.54%), 5p (27.72%), 5q (0.54%), 6p (0.54%), 7p (2.72%), 7q (0.54%), 8p (0.54%), **8q (36.96%)**, 9q (3.80%), 10p (0.54%), 10q (0.54%), 11q (1.08%), 12p (3.26%), 16p (1.63%), 16q (0.54%), 17q (0.54%), 18p (3.80%), 18q (0.54%), 19q (0.54%), 20p (1.08%), 20q (2.17%)
SKMC	1q (14.44%), 3p(1.11%), 3q (2.22%), 4p (3.33%), 5p (10.0%), **6p (25.56%)**, 7p (2.22%), 7q (2.22%), **8q (25.56%)**, 9p (1.11%), 9q (1.11%), 10p (1.11%), 11p (1.11%), 11q (2.22%), 12p (4.44%), 12q (1.11%), 16p (3.33%), 17p (2.22%), 17q (8.89%), 18p (2.22%), 18q (2.22%), 19p (1.11%), 19q (2.22%), 20q (7.78%), Xq (1.11%)
THCA	1q (1; 50.00%), 17q (2; 100.00%)

Bold indicates most common isochromosomes.

In isochromosomes, one whole-arm of a chromosome is expected to result in monosomy and the other whole-arm of the chromosome becomes trisomic, having 2 identical arms (Figure 4A). However, not all isochromosomes demonstrate monosomy. Interestingly, we observed whole-arm (Figure 4B) or segmental (Figure 4C) aUPD in the lost arm of isochromosomes, in which the deleted arm is duplicated, resulting in partial or whole-chromosomal arm acquired uniparental disomy instead of monosomy (Supplemental Fig. S1). We also observed i5p and segmental aUPD in chr5q in NCI H209 lung cancer cell line, which previously i5p was shown in karyotyping analysis [18] (Supplemental Fig. S2). Whole-chromosome arm (13.02%) and segmental (12.24%) aUPDs were observed in a total of 1,405 isochromosomes across the 11 tumor types. This finding suggests that loss of whole-chromosome arms or segments are frequently followed by reduplication in the following cell divisions. To support the hypothesis that whole-chromosome arm aUPD is a consequence of isochromosome, we compared whole-chromosome arm and segmental aUPD between isochromosomes (Figure 4B,C) and simple whole-chromosome arm deletion (Figure 4D) in recurrent isochromosomes (Figure 4E,F). We found that the frequency of whole-chromosome arm aUPD was significantly higher in the deleted arm of isochromosomes (Figure 4B) than in simple
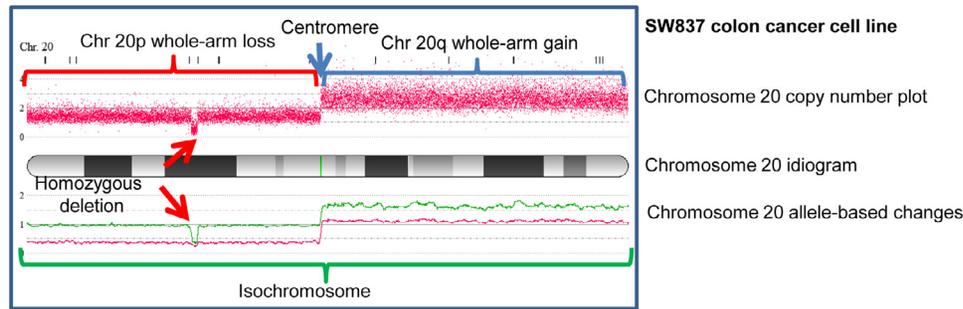
Figure 3. Isochromosome 20q in SW837 colon adenocarcinoma cell lines.
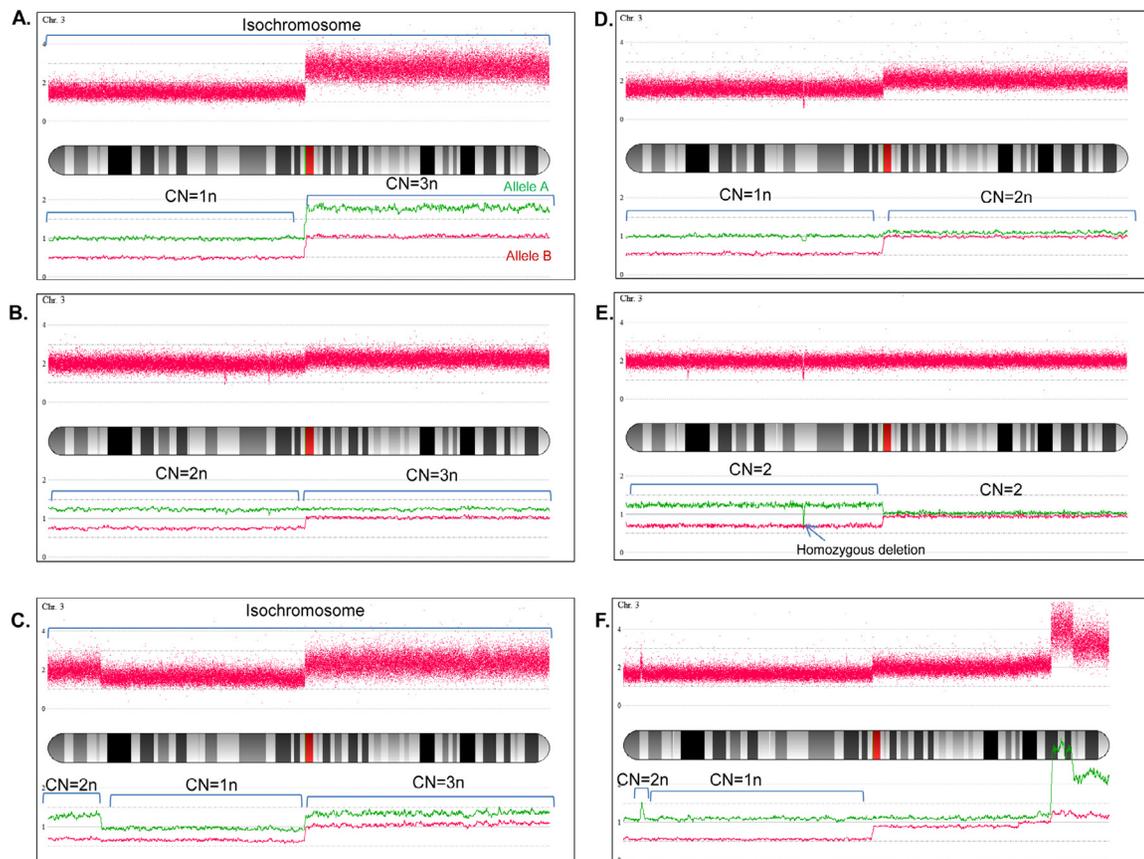
Figure 4. Representative figure for isochromosome, aUPD and simple chromosome arm deletion. These figures are representative of the data that are analyzed and reflect a mixture of normal and mutated cells (A) isochromosome 3q, (B) isochromosome 3q with whole-chromosome arm aUPD at 3p, (C) isochromosome 3q with segmental (telomeric) aUPD in chromosome 3p, (D) simple whole-chromosome arm deletion at 3p without trisomy in 3q arm, (E) whole-chromosome arm aUPD at simple whole-chromosome arm deleted 3p, (F) segmental aUPD at simple whole-chromosome arm deleted 3p.

deleted whole-chromosome arms (Figure 4E) ($p = 3.65$E-04, $q = 7.30$E-04), while no statistically significant difference in segmental aUPD was detected between deleted arms of isochromosomes (Figure 4C) and a simple deleted arm of chromosome (Figure 4F) ($p = 0.61$, $q = 0.61$) (Figure 5). This latter observation indicates that whole-chromosome arm aUPD is a frequent consequence of the development of isochromosomes. Moreover, we analyzed genotyping data from matching primary and recurrent tumor or primary and metastasis samples, and found that deleted regions were partially duplicated in primary tumor, and the transition to aUPD was completed or nearly completed in recurrent (Figure 6) and metastasis (Figure 7) tumors. This data indicates that, some deleted regions are partially repaired, and subsequent cell divisions are completed or nearly completed due to ongoing

repair. Thus aUPD is more likely the consequence of error prone repair regions.

We used a 10% mutation rate as threshold to find commonly mutated genes among tumors that contained isochromosomes. Four genes, *MUC16, FLG, SYNE1,* and *TTN* were mutated in at least 10% of samples with isochromosomes in all 11 cancer types. Two genes, *TP53* (except cervical cancers) and *RYR2* (except ovarian cancers) were mutated in samples with isochromosomes across 10 tumor types. *LRP1B* and *DST* (except colon and ovarian cancers), *CSMD3* (except cervical and rectal cancers), *PCLO* (except ovarian and cervical cancers), *ZFHX4* (except ovarian and rectal), and *CSMD1* (except ovarian and HNSC cancers) were mutated in 9 tumor types, *PCDH15* (except ovarian, cervical and esophageal cancer) was mutated

Figure 5. Number of samples with recurrent isochromosomes, aUPD and simple whole-chromosome arm deletion. (A) Number of samples with most common isochromosomes, whole-chromosome arm and segmental aUPD in deleted arm of isochromosomes in 11 type of tumors. (B) Number of samples with simple whole-chromosome arm deletion, whole-chromosome arm and segmental aUPD in simple deleted whole-chromosome arm in 11 type of tumors. (C) Summary figure for isochromosomes, aUPD and simple whole-chromosome arm deletion. ISO; isochromosomes, ISO+WCA aUPD; whole-chromosome arm aUPD in deleted arm of isochromosomes, ISO+S aUPD; segmental aUPD in deleted arm of isochromosomes, WGA DEL; simple whole-chromosome arm deletion, WCA DEL+WCA aUPD; whole-chromosome arm aUPD in simple whole-chromosome arm deletion, WCA DEL+S aUPD; segmental aUPD in simple whole-chromosome arm deletion.

Figure 6. Deletion in chromosome 17p and 17q (A) and chromosome 18 (B) in sample with primary lung adenocarcinoma. aUPD regions in chromosome 17p and 17q (C), and chromosome 18p (D) in same patients recurrence.

Figure 7. Chromosome 8 from the same patient's primary (A) and metastasis (B) sample. aUPD was observed in metastasis while deletion was observed in the same region in primary tumor.

in 8 tumor types. Since cohesin complex [(*SMC* (structural maintenance of chromosomes) (*SMC1a* or *SMC1b* and *SMC3*), *STAG* (*STAG1* or *STAG2* or *STAG3*), and *RAD21*], subunits and associated proteins *(MAU2, NIPBL, WAPL, PDS5A, PDS5B, CDCA5* and *REC8)* play a crucial role in proper chromosome segregation, we investigated whether cohesin gene mutations are also present in samples with isochromosomes, and found that mutations in cohesin genes are relatively common in this scenario (Supplemental Fig. 3). One or multiple SMC genes (SMC1A, SMC1B, SMC2, SMC3, SMC4, SMC5, SMC6) were mutated at least in 10% of samples with isochromosomes in all 11 types of tumors (Supplemental Table S2). However, the frequency of isochromosomes varied between the samples with and without mutations in *TP53, TTN* and *ZFHX4*. Isochromosomes were significantly higher in samples with *TP53* mutations than samples without mutations in 6 tumor types; lung squamous cell carcinoma ($p = 5.70E-04$, $q = 7.99E-03$), lung adenocarcinoma ($p = 2.86E-04$, $q = 4.01E-03$), head and neck squamous cell carcinoma ($p = 5.56E-10$, $q = 7.79E-09$), uterine cancers ($p = 1.61E-08$, $q = 2.26E-07$), colon ($p = 2.42E-05$, $q = 3.38E-04$) and stomach ($p = 2.44E-04$, $q = 3.42E-03$) cancers. Moreover, frequency of isochromosomes was significantly higher in samples with *TTN* ($p = 0.007$, $q = 0.034$), and *ZFHX4* ($p = 0.004$, $q = 0.025$) mutations compared to wild types in lung adenocarcinomas (Figure 8, Supplemental Table S2). To determine whether frequency of the mutated genes was increased in samples with isochromosomes, we performed Pearson chi-squared test comparing mutations in tumors with isochromosomes and those without isochromosomes and found mutations in the *TP53* gene were associated with the presence of isochromosomes in lung squamous cell carcinoma ($p = 0.002$, $q = 0.03$), lung adenocarcinoma ($p = 0.001$, $q = 0.015$), uterine ($p < 0.0001$, $q < 0.0001$), stomach ($p < 0.0001$, $q < 0.0001$), colon ($p < 0.0001$, $q < 0.0001$), and HNSCC ($p < 0.0001$, q < 0.0001). Interestingly, mutations in SMC genes were associated with samples with isochromosomes only in colon cancer ($p = 0.028$, $q = 0.047$). However, when we tested cohesion complex core components and regulatory genes together,

we found significant association between mutations in cohesion complex genes and samples with isochromosomes in colon ($p = 0.005$, $q = 0.019$) and stomach cancers ($p = 0.005$, $q = 0.038$). In addition, mutations at *ZFHX4* in LUAD ($p = 0.002$, $q = 0.015$), *MUC16* ($p < 0.0001$, q < 0.0001), *DST* ($p = 0.008$, $q = 0.024$), *CSMD3* ($p = 0.022$, $q = 0.041$), *PCLO* ($p = 0.015$, $q = 0.0375$), *LRP1B* ($p = 0.002$, $q = 0.01$) were associated with the presence isochromosomes in colon cancer.

Discussion

In this study we observed isochromosomes as a common event in 11 of the 12 solid tumor lineages examined except thyroid cancers. Interestingly, i8q was the one of the most frequent isochromosome in multiple types of tumors including HGSOV, uterine, gastric, colon, rectal, lung squamous cell, head and neck squamous cancers, lung adenocarcinomas and melanoma, and i5p was also common in 6 different types of tumors; HGSOV, cervical, esophageal, gastric, lung squamous cell and lung adenocarcinomas, while i3q was common in 5 tumor types; cervical, uterine, head and neck, esophageal and lung squamous cell carcinomas. Interestingly, i6p was frequent only in melanoma. The frequency of isochromosomes is higher in lung squamous cell carcinoma compared to lung adenocarcinoma and has a different pattern with the most common isochromosomes in LUSC being i3q and i5p, and i5p and i8q in LUAD. Besides, we found i20q in SW837 cell lines that previously was reported in karyotype analysis [17]. Isochromosomes have been proposed to be the most common structural alterations observed in solid tumors, and in particular in squamous cell carcinomas [21,22]. Thus our computational analysis is consistent with previous reports [17,18,21,22]. These data are compatible with isochromosomes and the processes leading to formation of isochromosomes contributing to the development of epithelial tumors. Isochromosomes 6p in retinoblastoma also were reported [23,24]. For reasons that are unknown, isochromosomes are extremely rare in thyroid cancers.
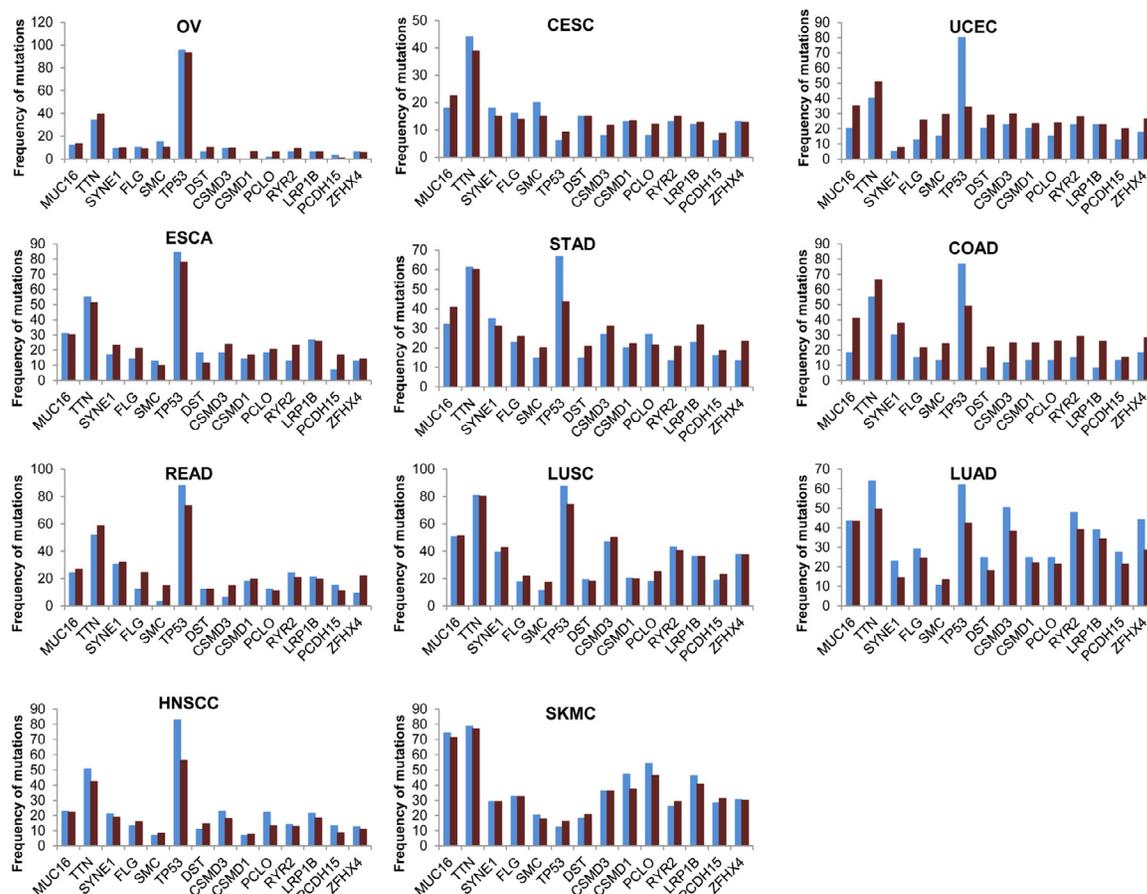
Figure 8. Frequency of mutations in 14 genes in samples with and without isochromosomes in 11 type of tumors. Blue bars represent samples with isochromosomes, and dark red bars represent samples without isochromsomes. Regardless of mutation numbers in an each gene, mutated gene was counted as positive, and non-mutated gene was considered as negative.

The selective presence of isochromosomes in different cancers supports the contention that formation of isochromosomes is not a random event.

An isochromosome normally results in decreased gene dosage in all genes in the deleted chromosome arm, and increased gene dosage in the duplicated chromosome arm, while simple whole-chromosome arm deletion leads to decreasing gene dosage in the lost arm of the chromosome. However, duplication of the deleted isochromosome arm in subsequent cell cycles can result in reconstitution of gene dosage by a subsequent aUPD. The aUPD could be associated with genes with decreased or increased activity resulting in difference in gene functionalities in the aUPD region due to existing alterations in the regions.

Several mechanisms of isochromosome formation have been proposed. Darlington [25] proposed that isochromosomes formation occurs in mitosis or meiosis through a misdivision of the centromere where instead of the centromere dividing longitudinally and separating sister chromatids, the centromere divides transversely separating the p- and q- arms of the chromosome. This results in an abnormal chromosome with identical arms due to loss of one arm, and trisomy of the remaining arm. Subsequent changes in gene dosage with for example oncogenic mutations being increased and tumor suppressor genes being decreased could contribute to cancer initiation and progression and/or therapy resistance.

When we studied recurrent mutations in tumor samples with isochromosomes, we found that 4 genes; *MUC16, FLG, SYNE1,* and *TTN* were selectively mutated at least in 10% of samples with isochromosomes across 11 tumor types. SMC genes are also mutated at least in 10% of samples with isochromosomes across 11 types of tumors. *TP53* and *RYR2*

are mutated at least 10% of samples with isochromosomes across 10 tumor types. The frequency of isochromosomes was significantly different between samples with and without *TP53* mutations in 6 tumor types. Moreover, *LRP1B, DST, CSMD3, PCLO,* and *CSMD1* were mutated at least 10% of samples with isochromosomes across 9 tumor types, and *PCDH15* was mutated across 8 tumor types. Also, isochromosomes were more common in samples with *ZFHX4* mutation compared to samples without mutations. We also found association between samples with isochromosomes and mutations in *TP53* and in 6 tumor types, and with mutations in core components of cohesion complex and regulatory genes in 2 tumor types, and with mutations at *SMC* genes, *ZFHX4, MUC16, DST CSMD3, PCLO,* and *LRP1B* in one cancer type. Thus combinations of mutations at *TP53* with cohesion complex genes may contribute to formation of isochromosomes. We have not ruled out the possibilities that some other genes we have not tested may contribute to formation of isochromosomes. The tumor suppressor p53 is known to be involved in DSB-triggered homologous recombination [26] with inactivation of p53 increasing the rate of homologous recombination [27], and isochromosome formation [28]. The SMC gene family encodes condensing complexes, and is essential for proper chromosome segregation, maintenance of chromosomal stability, DNA repair, development, genome integrity, and it is part of the cohesion complex [29]. SMC1 and STAG1/2 are core components of the cohesin complex that is involved in a variety of cellular functions including DNA damage repair [29,30]. Indeed, SMC1 and RAD21 contributes the repair of ionizing radiation-induced DNA DSB in the G2 phase of the cell cycle [31]. SYNE1 also has a role in DSB repair by facilitating gene conversion

[32]. Taken together, these mutated genes may also contribute to error prone repair of lost segments or whole-chromosome arms.

Next we found partial or whole-chromosome arm aUPD in deleted arms of isochromosomes. Interestingly, whole-chromosome aUPD is more frequent in deleted arms of isochromosome than in chromosomes with simple whole-chromosome arm deletion. The observation suggests that whole-chromosome arm aUPD occurs subsequent to loss of the arm of an isochromosome with whole-chromosome arm aUPD generated as part of the repair process of the deleted arm of an isochromosomes. Although homologous chromosomes serve as the template for recovery of the deleted chromosome arm in many cases an aberrant allele has the potential to be selected. Thus, aUPD could be a response to DNA damage and could pinpoint chromosome regions that are susceptible to DNA damage and error prone DNA repair. While we have demonstrated that whole-chromosome arm aUPD in cancer development is a consequence of isochromosome formation in many if not the majority of cases, there may be cases where alternative mechanisms lead to aUPD.

We also found segmental aUPD to have a similar frequency in deleted arms of isochromosomes as well as in chromosomes that underwent simple chromosome arm deletion. Previously it was proposed that segmental aUPD formation was a consequence of mitotic recombination [13,14]. However, we observed aUPD regions in recurrent and metastatic tumors that the same regions were deleted in matching primary tumors. Our data indicates that segmental aUPDs may occur as a consequence of repair processes in addition to mitotic recombination. Array based genotyping does not support direct visualization of chromosomal structure, but it does provide information about homo- and heterozygosity and the number of copies of each allele, chromosomal arm or segment in whole genome. Further studies would help to better understand what mechanisms lead to the observations of isochromosomes organization.

We propose a new mechanism that formation of aUPD is the result of a repair mechanism that cancer cells use to rescue the deleted fragment of a chromosome (Figs. 6 and 7), whole-chromosome arm or whole-chromosome. In the case were the homologous chromosome carries an aberrant oncogene or a tumor suppressor recovery leading to aUPD could result in homozygosity for existing alterations. In summary, the significant portion of whole-chromosome arm deletions is due to isochromosomes formation. The data presented in this study support a new 'break and copy' mechanism of isochromosomes formation, in which after a DSB induces a broken or a lost arm, complete or partial duplication and recovery of broken or lost arm of the chromosome could result in duplication of the lost arm by using the remaining allele and promulgation of the effects on any defects in the remaining allele.

CRediT authorship contribution statement

Musaffe Tuna: Conceptualization, Investigation, Supervision, Formal analysis, Writing original draft, Data interpretation, Writing review & editing. **Christopher I. Amos:** Data interpretation, Investigation, Writing review & editing. **Gordon B. Mills:** Conceptualization, Data interpretation, Investigation, Writing review & editing.

Declaration of Competing Interest

The authors declare no competing interests.

Acknowledgments

Ethics approval and consent to participate

In this research, we used The Cancer Genome Atlas (TCGA) generated data. TCGA Ethics & Policies was originally published by the National Cancer Institute (https://cancergenome.nih.gov/abouttcga/policies/informedconsent).

Data availability

All data are available from the GDC archieve (https://portal.gdc.cancer.gov) and XENA (https://xenabrowser.net).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.neo.2021.12.009.

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Further reading

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