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Tetraploidy in *BRCA2* breast tumours

Asta Bjork Jonsdottir [a], *Olafur Andri Stefansson* [a], *Johannes Bjornsson* [b,c], *Jon G. Jonasson* [b,c,d], *Helga M. Ogmundsdottir* [a,b], *Jorunn E. Eyfjord* [a,b,*]

[a] *Cancer Research Laboratory, Faculty of Medicine, University of Iceland, Reykjavik, Iceland*
[b] *Faculty of Medicine, University of Iceland, Reykjavik, Iceland*
[c] *Department of Pathology, Landspitali University Hospital, Reykjavik, Iceland*
[d] *Icelandic Cancer Registry, Icelandic Cancer Society, Reykjavik, Iceland*

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ABSTRACT

Tetraploidy and aneuploidy can be caused by cell division errors and are frequently observed in many human carcinomas. We have recently reported delayed cytokinesis in primary human fibroblasts from *BRCA2* mutation carriers, implying a function for the BRCA2 tumour suppressor in completion of cell division. Here, we address ploidy aberrations in breast tumours derived from *BRCA2* germline mutation carriers. Ploidy aberrations were evaluated from flow cytometry histograms on selected breast tumour samples ($n = 236$), previously screened for local *BRCA* mutations. The ploidy between *BRCA2*-mutated ($n = 71$) and matched sporadic ($n = 165$) cancers was compared. Differences in ploidy distribution were examined with respect to molecular tumour subtypes, previously defined by immunohistochemistry on tissue microarray sections. Tetraploidy was significantly 3 times more common in *BRCA2* breast cancers than sporadic. However, no differences were found in the overall ploidy distribution between *BRCA2*-mutation carriers and non-carriers. In *BRCA2* cancers, tetraploidy was associated with luminal characteristics. The increased frequency of tetraploidy in *BRCA2* associated cancers may be linked to cell division errors, particularly cytokinesis. Additionally, tetraploidy emerges predominantly in *BRCA2* breast cancers displaying luminal rather than triple-negative phenotypes.

1. Introduction

Inherited mutations in the breast cancer susceptibility genes *BRCA1* and *BRCA2* increase the risk of breast cancer as well as ovarian, prostate and other types of cancers.[1] For carriers of the Icelandic founder mutation *BRCA2 999del5*, the risk translates into over 70% by the age of 70.[2] The increased susceptibility has been attributed to functions of the BRCA proteins in repair of DNA double strand breaks, via homologous recombination. Defects in the repair mechanisms can alter recombination pathways, giving rise to chromosomal re-arrangements, which have been implicated as the main cause for chromosomal instability found in the tumours of *BRCA2* mutation carriers.[3,4] More recently, it has been suggested that the BRCA2 protein functions in cytokinesis.[5,6] Defects in daughter cell separation can cause mis-segregation of chromosomes, giving rise to polyploidy[7] and could therefore contribute to the increased cancer susceptibility in *BRCA2*-mutation carriers.

Genomic alterations are common in breast tumours. Variations in gene expression patterns can be accompanied by corresponding phenotypic diversity of breast tumours. Breast carcinomas can arise from at least two distinct cell types:

* *Corresponding author:* Address: Cancer Research Laboratory, Faculty of Medicine, University of Iceland, Vatnsmyrarvegi 16, 101 Reykjavik, Iceland. Tel.: +354 5255825.
E-mail address: jorunne@hi.is (J.E. Eyfjord).
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basal-like and luminal epithelial cells, and can be categorised into five subtypes based on expression of three biomarkers, oestrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2). Better definition can be achieved by adding epidermal growth factor receptor (EGFR) and cytokeratin 5/6 (CY5/6) expression analysis.[8,9] The five subtypes of breast tumours are luminal A and luminal B (positive for either ER or PR), HER2 overexpressing (ER and PR negative), normal-like and basal-like, including triple-negative phenotype (TNP) for ER, PR and HER2. The basal-like subtype can further be subdivided into five-marker negative phenotype (5NP) or Core Basal.[10] *BRCA2* tumours are predominantly luminal A or luminal B.[11,12] They show frequent and complex genomic alterations[13,14] and have relatively large deletions and few high-level amplifications.[14]

A potential function for the BRCA2 protein in cytokinesis has been shown in modified mouse embryonic fibroblasts and HeLa cells.[5] We have recently reported that BRCA2 plays a role in the process in primary human fibroblast cells.[6] It is well known that unsuccessful cell division can cause incomplete chromosomal segregation, gives rise to altered ploidy and supernumerary centrosomes.[7,15] Tetraploidy can emerge through various mechanisms, such as cell fusion, mitotic slippage, and failure to undergo cytokinesis. A failure to undergo cytokinesis may occur because of a failure or disturbance of cleavage furrow formation, giving rise to a new G_1 bi-nucleated cell with two centrosomes.[16,17] It has been proposed that tetraploid cells represent an important intermediate on the route to aneuploidy.[7] Active checkpoints prevent tetra- and aneuploidisation. They act to ensure accurate cell division and inhibit cycling of cells that have not exited mitosis efficiently.[18,19]

There is an indication that *BRCA2*-mutation status is associated with bi-nucleation and amplification of centrosomes.[20] Mouse cells deficient for *Brca2* produce polyploid cells.[21] The majority of the Icelandic *BRCA2 999del5* breast cancer cases have been shown to be non-diploid.[3] A study presenting an association between *BRCA2* mutation and high grade tumour progression additionally to increased proliferation rate, also found that non-diploidy was of higher frequency in *BRCA2*-mutated tumours than sporadic.[22]

The aim of this study was to investigate the proportion of tetraploidy and aneuploidy in *BRCA2*-mutated breast tumours, as a potential consequence of cytokinesis defects. We compared the DNA content in *BRCA2*-mutated and sporadic breast tumours, measured with flow cytometry. Ploidy aberrations were then linked to molecular classification of the tumours.

2. Material and methods

2.1. Patient samples

Malignant breast tumour samples were selected from a patient cohort that had previously been screened for local *BRCA* germline mutations and had been diagnosed from 1990–2006. Fulfilling these criteria were 101 *BRCA2*-mutation carriers. Two hundred and two sporadic cases that matched for year and age at diagnosis were selected as controls. Flow cytometry data for ploidy evaluation were available for 71 tumours from selected *BRCA2*-mutation carriers and 165 from sporadic controls.

All *BRCA2*-mutated samples carry an Icelandic founder mutation, *BRCA2 999del5*.[23] Exon 9 of the *BRCA2* gene was PCR-amplified from peripheral blood DNA and run on 7.5% polyacrylamide gels for detection of the *999del5* mutation.[23,24]

This work was carried out according to permission from the Icelandic Data Protection Commission (2006050307) and National Bioethics Committee (VSNb2006050001/03-16). Informed consent was obtained from all patients.

2.2. Ploidy data

Flow cytometry analysis has been performed routinely on invasive breast tumour samples at the Department of Pathology, Landspitali University Hospital, Reykjavik, Iceland, since 1990. Flow cytometry histograms, indicating DNA content, were classified as diploid when they showed a single G_0/G_1 peak. Samples with at least two clear distinct peaks were classified as aneuploid. When the ratio was 1.80:2.10, and therefore the DNA content in the cells in the second peak was about twice as much as in the normal peak, the sample was classified as tetraploid. Tumours containing both aneuploid and tetraploid cells were classified as mixed aneu- and tetraploid. DNA ploidy index and S-phase assessment were performed and classified as described.[25] Only one DNA content measurement from each tumour was included.

The S-phase value of <7% was defined as low S-phase, and $\geqslant$7% was defined as high S-phase.

2.3. Immunohistochemistry and tissue microarray

For molecular characterisation of the breast tumour samples immunohistochemistry was performed on tissue microarray sections. Expression analysis was carried out for ER, PR, HER-2, EGFR, CK5/6, CK8, CK18 and BRCA1, as described earlier.[14,26]

2.4. Statistical analysis

Statistical analysis was carried out using SPSS 17.0 and R 2.10.1. Associations between the *BRCA2* mutation status of the breast tumours and ploidy, as well as between the phenotype of tumours and ploidy in *BRCA2*-mutated and sporadic samples were calculated using χ^2-tests.

3. Results

3.1. Ploidy aberrations of BRCA2 and sporadic breast tumours

Ploidy was examined in a selected set of 236 breast tumours derived both from *BRCA2*-mutation carriers and sporadic breast cancer cases. The frequency of diploidy, aneuploidy, tetraploidy and mixed aneu- and tetraploidy was compared between *BRCA2* and sporadic tumours.

Table 1 summarises the ploidy distribution of sporadic and *BRCA2*-mutated tumours showing no differences in the overall distribution between the two groups (χ^2 test statistic 6.18, *P*-value 0.103). Nearly half of both sporadic and *BRCA2* tumours were diploid (47.9% and 42.3%, respectively).

Table 1 – Comparison of ploidy between *BRCA2* and sporadic cancers.

	Diploid (%)	Aneuploid (%)	Tetraploid (%)	Mixed Aneu- and Tetraploid (%)	Total
Sporadic	79 (47.9)	63 (38.2)	8 (4.8)	15 (9.1)	165 (100%)
BRCA2-mutated	30 (42.3)	24 (33.8)	10 (14.1)	7 (9.9)	71 (100%)
Total	109	87	18	22	236

χ^2 test statistic 6.18, *P*-value 0.103.

Although the overall distribution was the same, tetraploidy was significantly more common in *BRCA2* breast tumours than sporadic. The difference was almost 3-fold, 14.1% (10 of 71) as compared with 4.8% (8 of 165) (χ^2 test statistic 4.77, *P*-value 0.029) (Fig. 1).

The percentage of cells in S-phase of the cell cycle is an indicator of the proliferation rate of a tumour. The S-phase in *BRCA2* tumours was higher than in sporadic tumours (χ^2 test statistic 3.71, *P*-value 0.054). High S-phase was associated with polyploidy both in *BRCA2*- (χ^2 test statistic 32.24, *P*-value <0.001) and sporadic tumours (χ^2 test statistic 47.03, *P*-value <0.001) (Additional file Table 1).

3.2. *Molecular classification of BRCA2 and sporadic breast tumours in relation to ploidy*

Breast cancers are classified into subtypes based on their molecular profiles. Expression data for ER, PR and HER2, analysed by immunohistochemistry (IHC) on tissue microarrays, were available from another study[26] for a subset of the study group (52 *BRCA2* and 43 sporadic cancers). This allowed us to examine ploidy aberrations in relation to molecular phenotypes established through a validated classification scheme.[10]

All of the *BRCA2* cancers sub-categorised as either luminal (37 of 52, 71.1%) or TNP (15 of 52, 28.9%) as none displayed HER2 overexpression. From Table 2 and Fig. 2 it is clear that within the group of *BRCA2* cancers the differences between luminal and TNP subtypes are reflected in the type of ploidy abnormalities, and the association between ploidy aberrations and molecular subtype was highly significant (χ^2 test statistic 18.48, *P*-value <0.001). All of the 7 tetraploid *BRCA2* cancers and 5 out of the 6 mixed aneu- and tetraploid belonged to the luminal phenotype (χ^2 test statistic 8.65, *P*-value 0.003) (Fig. 2).

In contrast, all except one of the TNP *BRCA2* tumours displaying ploidy aberrations were aneuploid only (11 of 12, 91.7%) (Table 2).

The group of sporadic cancers also showed a statistically significant association between subtype and ploidy (χ^2 test statistic 20.47, *P*-value 0.015) (Table 2), as those of the luminal phenotype were mostly diploid or aneuploid, with only one out of 29 being tetraploid (Table 2). Although the numbers are very low they do not suggest the association between luminal phenotype and tetraploidy that was seen in the *BRCA2* group.

4. Discussion

The results presented here describe an analysis of ploidy in cancers derived from *BRCA2* germline mutation carriers and sporadic cancers. The study was carried out to follow up on our previous report describing a function for BRCA2 in the completion of cell division of primary human cells.[6] We observed that tetraploidy was significantly more frequent in

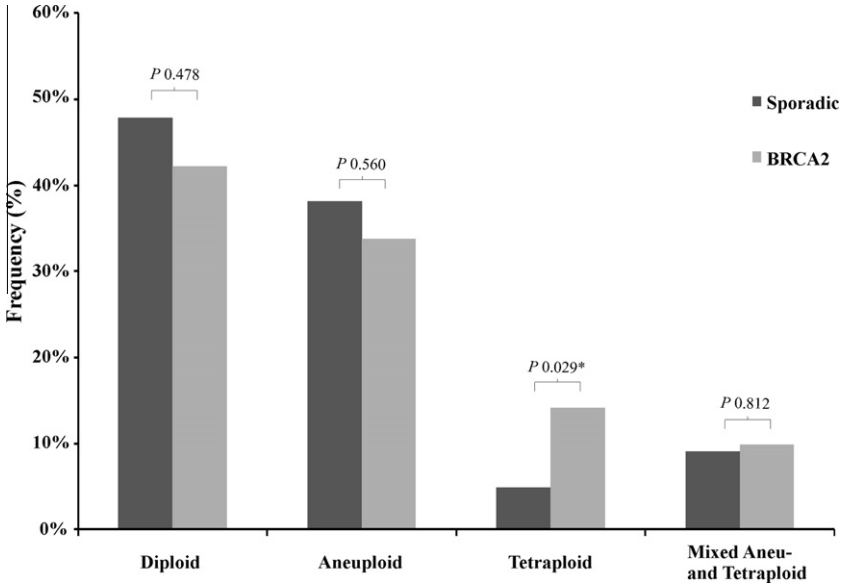

Fig. 1 – Ploidy distribution of sporadic (dark grey) and *BRCA2* (light grey) breast cancers. The proportion of diploidy, aneuploidy and mixed aneu- and tetraploidy was similar in sporadic and *BRCA2* cancers. Tetraploidy was 2.9-fold higher in *BRCA2* cancers compared with sporadic cancers (*P*-value 0.029), see details in Table 1.

Table 2 – Ploidy of *BRCA2* and sporadic cancers in relation to molecular subtypes.

	Diploid (%)	Aneuploid (%)	Tetraploid (%)	Mixed Aneu- and Tetraploid (%)	Total
BRCA2-mutated					
Luminal	20 (54.1)	5 (13.5)	7 (18.9)	5 (13.5)	37 (100%)
TNP	3 (20.0)	11 (73.3)	0 (0.0)	1 (6.7)	15 (100%)
Total	23	16	7	6	52
χ^2 test statistic 18.48, *P*-value <0.001.					
Sporadic					
Luminal	17 (58.6)	11 (37.9)	1 (3.4)	0 (0.0)	29 (100%)
TNP	1 (12.5)	4 (50.0)	1 (12.5)	2 (25.0)	8 (100%)
Luminal-HER	0 (0.0)	1 (25.0)	1 (25.0)	2 (50.0)	4 (100%)
Non-Luminal-HER2	1 (50.0)	1 (50.0)	0 (0.0)	0 (0.0)	2 (100%)
Total	19	17	3	4	43
χ^2 test statistic 20.47, *P*-value 0.015.					

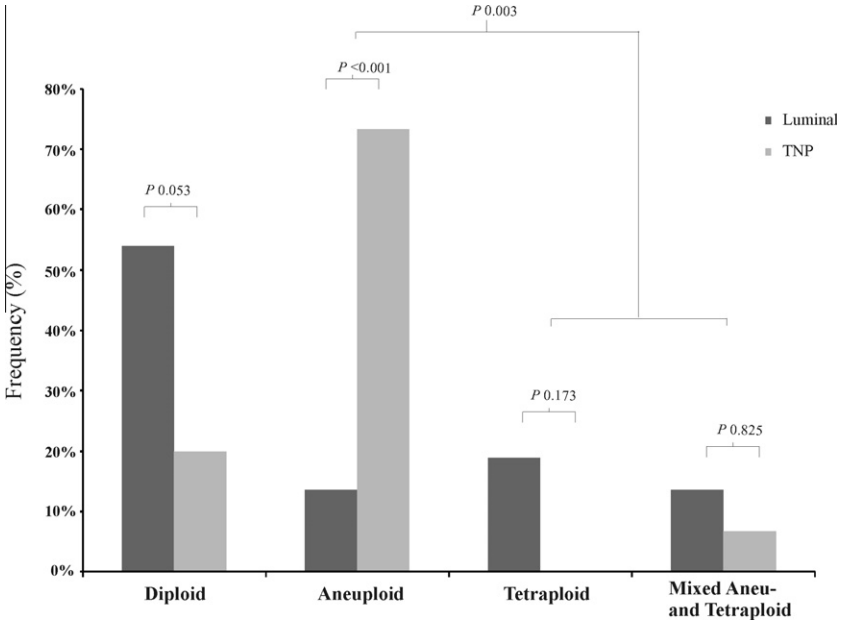

Fig. 2 – Ploidy distribution of *BRCA2* breast cancers, in relation to molecular subtype. Dark grey bars represent luminal subtype and light grey triple-negative phenotype (TNP). A strong association was seen between luminal phenotype and tetraploidisation (only tetraploidy or mixed aneu-and tetraploidy) (*P*-value 0.003), and TNP and aneuploidy (*P*-value <0.001) in *BRCA2* cancers. TNP was rarely diploid.

BRCA2-mutated than sporadic breast carcinomas. Furthermore, our results showed that tetraploidy in *BRCA2* cancers was almost confined to tumours of a luminal subtype.

When studying tetraploidy specifically, a 3-fold difference between *BRCA2*-mutated and sporadic cancers was detected, but tetraploidy for *BRCA2* cancers has not been described previously. There was no overall association between *BRCA2* status and ploidy, and this is in line with previously published data.[3,22] Molecular classification was available for a subset of the studied tumour samples.[26] Tetraploid *BRCA2*-mutated cancers displayed luminal- rather than triple-negative phenotype. This is in contrast with that observed in sporadic cancers of luminal phenotype, not HER-2 related, which were mostly diploid or aneuploid and rarely showed tetraploid features.

Recently, it has been proposed that the wild-type *BRCA2* allele is not consistently lost in breast tumours from *BRCA2* mutation carriers.[26,27] Ongoing studies indicate that deletion of the wild-type *BRCA2* allele may be more frequent in *BRCA2* cancers displaying luminal rather than triple-negative phenotype. Given a role for BRCA2 in cytokinesis, this might explain why tetraploidy emerges in luminal- rather than triple-negative *BRCA2* cancers.

Tetraploidy is known to arise through incomplete cytokinesis. By using time-lapse live cell imaging to estimate cell division time we found that *BRCA2*-heterozygous primary human fibroblasts showed delayed cytokinesis,[6] supporting a role for BRCA2 in this process.[5] The *BRCA2* heterozygous primary fibroblasts had a slightly increased number of cells in G_2/M phase.[6] Spontaneous tetraploidy has been observed in

cultured primary human skin fibroblasts derived from *BRCA2*-mutation carriers (Ogmundsdottir, unpublished data).

Previously established functions of the BRCA2 protein include an essential role in error-free repair of DNA double strand breaks via homologous recombination.[28] It is also needed at stalled replication forks,[29] and it may regulate mitotic entry to some extent.[30] Cytokinesis can fail because of defects in or deficiency of proteins mediating cytokinesis. Also, chromosome bridges formed during anaphase can cause furrow regression leading to generation of bi-nucleated cells.[31–33] End-to-end fusions of chromosomes are more common in breast tumours from *BRCA2* carriers than in sporadic breast tumours.[28,34,35] These chromosomes frequently contain two or more centromeres, which make segregation of sister-chromatids during mitosis difficult. The high degree of chromosomal re-arrangements and other genetic instability in *BRCA2*-deficient cell lines and tumours has been explained by inefficient repair of DNA double strand breaks, via error-prone non-homologous end joining, which can result in G_1 arrest. *BRCA2* mutations could therefore facilitate polyploidisation through cytokinesis failure as well as creation of chromosome bridges.

Polyploidy is not necessarily a disadvantage.[36] Only when growth arrest, cell death or increased genomic instability is triggered can it be catastrophic.[37] Thus, polyploidy can be tolerated so long as cells retain the cellular machinery to duplicate and segregate their genome and centrosomes accurately during subsequent divisions. Tetraploid cells do not always produce tetraploid progeny. Aneuploidy can evolve either through tetraploidy or directly from diploidy.[38] Tetraploid cells frequently contain two extra centrosomes. Only when polarity is maintained, by forming a bipolar spindle, can a normal cell division be executed. Amplification of centrosomes can cause multipolar spindles, resulting in unequal distribution of chromatids between the daughter cells, giving rise to aneuploid progenies.[15,39] The aneuploid and the mixed aneu- and tetraploid cell populations may have been promoted through the tetraploid rout, or they may have evolved directly from diploid cells, possibly as a consequence of multipolar spindle or spindle checkpoint failure, resulting in mis-segregation of chromosomes.

In conclusion, tetraploidy was more frequent in *BRCA2*-mutated tumours than sporadic. We have previously reported that *BRCA2* heterozygous cells have delayed cytokinesis. We suggest that the increased tetraploidy in the *BRCA2* tumours may be related to defects in cytokinesis. Tetraploidy was found to be associated with luminal phenotype in the *BRCA2* samples. Thus, it may be speculated that loss of the BRCA2 function is more prominent in *BRCA2* cancers displaying luminal characteristics rather than triple-negative.

Conflict of interest statement

None declared.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejca.2011.11.008.

REFERENCES

1. Miki Y, Swensen J, Shattuck-Eidens D, et al. A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. *Science* 1994;**266**:66–71.
2. Tryggvadottir L, Sigvaldason H, Olafsdottir GH, et al. Population-Based Study of Changing Breast Cancer Risk in Icelandic BRCA2 Mutation Carriers, 1920–2000. *J Natl Cancer Inst* 2006;**98**:116–22.
3. Gretarsdottir S, Thorlacius S, Valgardsdottir R, et al. BRCA2 and p53 mutations in primary breast cancer in relation to genetic instability. *Cancer Res* 1998;**58**:859–62.
4. Venkitaraman AR. Cancer susceptibility and the functions of BRCA1 and BRCA2. *Cell* 2002;**108**:171–82.
5. Daniels MJ, Wang YM, Lee MY, Venkitaraman AR. Abnormal cytokinesis in cells deficient in the breast cancer susceptibility protein BRCA2. *Science* 2004;**306**:876–9.
6. Jonsdottir AB, Vreeswijk MPG, Wolterbeek R, et al. BRCA2 heterozygosity delays cytokinesis in primary human fibroblasts. *Cell Oncol* 2009;**31**:191–201.
7. Storchova Z, Kuffer C. The consequences of tetraploidy and aneuploidy. *J Cell Sci* 2008;**121**:3859–66.
8. Perou C, Sorlie T, Eisen M, et al. Molecular portraits of human breast tumours. *Nature* 2000;**406**:747–52.
9. Chin K, DeVries S, Fridlyand J, et al. Genomic and transcriptional aberrations linked to breast cancer pathophysiologies. *Cancer Cell* 2006;**10**:529–41.
10. Cheang M, Voduc D, Bajdik C, et al. Basal-like breast cancer defined by five biomarkers has superior prognostic value than triple-negative phenotype. *Clin Cancer Res* 2008;**14**:1368–76.
11. Sorlie T, Tibshirani R, Parker J, et al. Repeated observation of breast tumor subtypes in independent gene expression data sets. *Proc Natl Acad Sci USA* 2003;**100**:8418–23.
12. Waddell N, Arnold J, Cocciardi S, et al. Subtypes of familial breast tumours revealed by expression, copy number profiling. *Breast Cancer Res Treat* 2009.
13. Tirkkonen M, Johannsson O, Agnarsson B, et al. Distinct somatic genetic changes associated with tumor progression in carriers of BRCA1 and BRCA2 germ-line mutations. *Cancer Res* 1997;**57**:1222–7.
14. Stefansson OA, Jonasson JG, Johannsson OT, et al. Genomic profiling of breast tumours in relation to BRCA abnormalities, phenotypes. *Breast Cancer Res* 2009;**11**:R47.
15. Gisselsson D. Classification of chromosome segregation errors in cancer. *Chromosoma* 2008;**117**:511–9.

16. Storchova Z, Pellman D. From polyploidy to aneuploidy, genome instability and cancer. *Nat Rev Mol Cell Biol* 2004;**5**:45–54.
17. Andreassen PR, Lohez OD, Lacroix FB, Margolis RL. Tetraploid state induces p53-dependent arrest of nontransformed mammalian cells in G1. *Mol Biol Cell* 2001;**12**:1315–28.
18. Meraldi P, Sorger PK. A dual role for Bub1 in the spindle checkpoint and chromosome congression. *EMBO J* 2005;**24**:1621–33.
19. Lanni JS, Jacks T. Characterization of the p53-dependent postmitotic checkpoint following spindle disruption. *Mol Cell Biol* 1998;**18**:1055–64.
20. Tutt A, Gabriel A, Bertwistle D, et al. Absence of Brca2 causes genome instability by chromosome breakage and loss associated with centrosome amplification. *Curr Biol* 1999;**9**:1107–10 [S1].
21. Lee H, Trainer AH, Friedman LS, et al. Mitotic checkpoint inactivation fosters transformation in cells lacking the breast cancer susceptibility gene, Brca2. *Mol Cell* 1999;**4**:1–10.
22. Agnarsson BA, Jonasson JG, Bjornsdottir IB, et al. Inherited BRCA2 mutation associated with high grade breast cancer. *Breast Cancer Res Treat* 1998;**47**:121–7.
23. Thorlacius S, Olafsdottir G, Tryggvadottir L, et al. A single BRCA2 mutation in male and female breast cancer families from Iceland with varied cancer phenotypes. *Nat Genet* 1996;**13**:117–9.
24. Gudmundsdottir K, Thorlacius S, Jonasson JG, et al. CYP17 promoter polymorphism and breast cancer risk in males and females in relation to BRCA2 status. *Br J Cancer* 2003;**88**:933–6.
25. Jonasson JG, Hrafnkelsson J. Nuclear DNA analysis and prognosis in carcinoma of the thyroid gland. *Virchows Arch* 1994;**425**:349–55.
26. Stefansson OA, Jonasson JG, Olafsdottir K, et al. Genomic and phenotypic analysis of BRCA2 mutated breast cancers reveals co-occurring changes linked to progression. *Breast Cancer Res* 2011;**13**:R95.
27. King T, Li W, Brogi E, et al. Heterogenic loss of the wild-type BRCA allele in human breast tumorigenesis. *Ann Surg Oncol* 2007;**14**:2510–8.
28. Patel KJ, Yu VPCC, Lee H, et al. Involvement of Brca2 in DNA Repair. *Mol Cell* 1998;**1**:347–57.
29. Lomonosov M, Anand S, Sangrithi M, Davies R, Venkitaraman AR. Stabilization of stalled DNA replication forks by the BRCA2 breast cancer susceptibility protein. *Genes Dev* 2003;**17**:3017–22.
30. Ayoub N, Rajendra E, Su X, et al. The carboxyl terminus of Brca2 links the disassembly of Rad51 complexes to mitotic entry. *Curr Biol* 2009;**19**:1075–85.
31. Ganem NJ, Storchova Z, Pellman D. Tetraploidy, aneuploidy and cancer. *Curr Opin Genet Dev* 2007;**17**:157–62.
32. Weaver BAA, Silk AD, Cleveland DW. Cell biology: Nondisjunction, aneuploidy and tetraploidy. *Nature* 2006;**442**:E9–E10.
33. Glotzer M. The molecular requirements for cytokinesis. *Science* 2005;**307**:1735–9.
34. Yu VP, Koehler M, Steinlein C, et al. Gross chromosomal rearrangements and genetic exchange between nonhomologous chromosomes following BRCA2 inactivation. *Genes Dev* 2000;**14**:1400–6.
35. Eyfjord JE, Bodvarsdottir SK. Genomic instability and cancer: networks involved in response to DNA damage. *Mutat Res* 2005;**592**:18–28.
36. Weglarz TC, Degen JL, Sandgren EP. Hepatocyte transplantation into diseased mouse liver. Kinetics of parenchymal repopulation and identification of the proliferative capacity of tetraploid and octaploid hepatocytes. *Am J Pathol* 2000;**157**:1963–74.
37. Weaver BA, Silk AD, Montagna C, Verdier-Pinard P, Cleveland DW. Aneuploidy acts both oncogenically and as a tumor suppressor. *Cancer Cell* 2007;**11**:25–36.
38. King RW. When 2 + 2 = 5: the origins and fates of aneuploid and tetraploid cells. *Biochim Biophys Acta* 2008;**1786**:4–14.
39. Borel F, Lohez OD, Lacroix FB, Margolis RL. Multiple centrosomes arise from tetraploidy checkpoint failure and mitotic centrosome clusters in p53 and RB pocket protein-compromised cells. *Proc Natl Acad Sci USA* 2002;**99**:9819–24.