Aneuploidy Is Associated with TP53 Expression but not with BRCA1 or TERT Expression in Sporadic Colorectal Cancer

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Abstract. *Background: Defective expression of genes involved in mitotic chromosome segregation (e.g. AURKA, BUB1B), DNA damage response (e.g. TP53, BRCA1), and telomere function (e.g. TERT) may play a role in the development of tumor aneuploidy. Materials and Methods: The levels of TP53, BRCA1 and TERT were assessed in 55 sporadic colorectal tumors and 37 normal mucosas using tissue microarrays and immunohistochemical detection, and their associations with DNA aneuploidy, levels of mitotic spindle proteins AURKA, AURKB, MAD2L1 and BUB1B and clinicopathological parameters were investigated. Results: DNA aneuploidy was associated only with TP53 alterations. BRCA1 expression in tumors was significantly correlated with individual mitotic spindle protein expressions, and TERT and MAD2L1 expressions were moderately correlated in the tumor group, suggesting a putative role for TERT in MAD2L1 regulation. Conclusion: Loss of TP53 function appears to be involved in the development of aneuploidy, but not in the deregulation of mitotic spindle protein function.*

Aneuploidy is defined as abnormal numerical and structural chromosomal content (1) and is a dominant cellular phenotype for the majority of sporadic colorectal carcinomas. Aneuploid colorectal tumors exhibit extensive chromosomal instability (CIN) (2-5), defined as an increased frequency of acquiring new structural and numerical chromosomal aberrations during proliferation (3). CIN and/or aneuploidy can be caused by the defective expression of genes involved in the following cellular processes: mitotic chromosome segregation (*e.g. AURKA, AURKB, BUB1B, MAD2L1*) (1, 6-13), DNA damage response (*e.g. BRCA1*) (14, 15), and telomere function (*e.g. TERT*) (16-19). Associations of TP53 alterations with aneuploidy have previously been demonstrated for sporadic colorectal cancer, suggesting that the loss of functional TP53 facilitates the formation and survival of cells with aneuploid DNA content since downstream DNA damage response, DNA repair, and apoptosis signaling pathways would not function normally (5, 20-21). We recently reported that reduced levels of BUB1B in sporadic colorectal carcinomas were significantly associated with aneuploidy (22). It was thus of interest to extend our studies of defective mitotic spindle protein expression to investigations of the expressions of other proteins reported to play a role in the development of aneuploidy/CIN, specifically BRCA1, TP53, and TERT, as well as to explore the potential associations of these proteins with AURKA, AURKB, BUB1B and MAD2L1 in sporadic colorectal cancer, especially in light of recent reports demonstrating roles for AURKA in the inactivation (23) and degradation of TP53 (24), in the phosphorylation of BRCA1 during the G_2/M transition (25), and in the induction of TERT expression/activity *via* up-regulation of MYC (V-myc myelocytomatosis viral oncogene homolog (avian)) (26). The levels of BRCA1, TP53, and TERT were assessed in a series of well-characterized sporadic colorectal carcinomas and examined for potential associations with tumor DNA aneuploidy, with each other, with relevant clinicopathological parameters, and with the expression of AURKA, AURKB, MAD2L1, and BUB1B proteins, as potential associations might provide some indications as to which proteins play a key role in the aneuploidization process, singly or in combination with other relevant proteins. The effects of mitotic spindle protein overexpression on the inactivation of BRCA1, TP53 and TERT in sporadic colorectal cancer were also of interest.

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Materials and Methods

Patient biopsies, clinicopathological features, and clinical outcome. Patient material consisted of 55 tumor resections taken from patients who had undergone attempted radical surgery for sporadic colorectal cancer during the period 1990-2001 at three regional hospitals (Ullevål University Hospital, Asker and Bærum County Hospital

and Vestfold County hospital). The patient group consisted of 30 males and 25 females, with a median age of 68 years (range 35-88 years). None of the patients received radiation or chemotherapy prior to surgery. Twenty tumor specimens contained peritumoral morphologically normal mucosa (proximal normal mucosa). Normal mucosas collected from colectomy specimens without cancer from an additional 17 patients were used as normal controls. The carcinomas were histologically classified as poorly (n=8), moderately (n=38) or highly (n=6) differentiated; 3 tumors showed mucinous differentiation. The degree of tumor spread was assessed using Dukes' staging: 3 tumors were classified as Dukes' A, 31 as Dukes' B, 13 as Dukes' C and 8 as Dukes' D. The majority of this tumor series was previously evaluated for ploidy status (5), gross chromosomal aberrations (5), levels of the mitotic spindle proteins AURKA, AURKB, MAD2L1 and BUB1B (22), and levels of proliferation using the Ki-67 antibody (22). Thirty-three (60%) out of the 55 tumors were classified as aneuploid and 22 (40%) as diploid using DNA flow cytometry (5). Forty-one percent, 51%, 53%, and 54% of the colorectal tumors studied in the present work were previously shown to overexpress AURKA, AURKB, MAD2L1, and BUB1B respectively (22). Details of patient survival at last follow-up (January 2007) were obtained from medical records subsequent to having obtained the necessary permission to use patient information according to regional ethical guidelines. Thirty-seven patients were deceased (mean survival time of 33 months) and 18 patients were still alive as of the follow-up date.

Tissue microarrays and immunohistochemistry for BRCA1, TP53 and TERT proteins. Preparation of tissue microarrays was described in detail in our previous report (27). The sporadic colorectal cancer microarrays utilized for the present study consisted of 3 cores of tumor tissue from each patient, 2 cores of proximal normal mucosa, and 2 cores of normal mucosa. All the core tumor specimens had previously been evaluated by an experienced pathologist (OPFC) as representative of the tumor block from which they were sampled. Tissue cores sampled from the proximal mucosa were localized to <5 mm from the cancer margin.

Xylene-dewaxed paraffin tissue microarray sections (4 microns thick) were exposed to 0.5% H_2O_2 solution for 10 min to block endogenous peroxidase, rinsed in tap water for 5 min, and subjected to antigen retrieval in EDTA buffer, pH 8.0 (BRCA1) and in citrate buffer, pH 6.0 (telomerase and TP53) as described previously (27). Tumor sections (as well as the tonsillar tissue biopsies that served as positive staining controls) were subsequently incubated with primary antibodies against telomerase (TRT (H-231), 1:50 dilution; Santa Cruz Biotechnology, Santa Cruz CA, USA), TP53 (Ab-2, 1:200 dilution; Oncogene Research Products, Cambridge MA, USA) and BRCA1 (1:20 dilution; Cell Signaling Technology, Danvers MA, USA) as described previously (27). Sections stained with Tris buffered saline (TBS) instead of primary antibodies functioned as negative staining controls. TP53 staining was performed on a Ventana Nexes machine using Ventana "*I*" view DAB Detection kit (Ventana Medical Systems, Tucson AZ, USA) according to the manufacturer's protocol. Three to four hundred cells per core were counted for each specimen (cancer, proximal normal mucosa or normal mucosa) for a total of circa 1,000 cells per patient. The mean percentage of positive cells ± standard deviation (SD) or the median percentage (including range) was calculated from the number of positive cells out of 1,000 cells counted. This percentage value (% positivity) defined the protein expression for each sample.

Statistical analyses. SPSS 15 (SPSS Inc., Chicago, IL, USA) and Prism 4 (GraphPad Software, La Jolla, CA, USA) were used for statistical testing and the generation of data tables and frequency distributions. The relationships between protein expression and clinicopathological features were evaluated using parametric or non-parametric contingency testing and correlation analyses as indicated. Overall survival was assessed using time-to-event analyses from the date of surgery to the time of last follow-up and visualized using Kaplan-Meier plots. A p-value <0.05 denoted statistical significance.

Results

Immunohistochemical assessments of BRCA1, TP53 and TERT levels in colorectal tumors and correlations with tumor parameters. Representative images of colorectal biopsies and normal mucosas from tissue microarrays immunohistochemically stained for BRCA1, TP53 and TERT are shown in Figure 1. Protein localizations were primarily nuclear for tumors and normal mucosas, although cytoplasmic staining could be seen in some TERT-stained tumors. For counting purposes, only positive nuclei were counted. Protein expression levels were defined as the number of cells (expressed as a percentage value) positive for a specific protein. Frequency distributions for BRCA1, TP53 and TERT expressions in the tumor group were parametric, non-parametric and non-parametric, respectively, and these were used to determine whether the mean (parametric distribution) or the median (non-parametric distribution) was used as the cut-off level of expression for statistical testing.

Table I shows the mean (BRCA1) and median (TP53, TERT) levels of expression for each of these proteins in normal mucosas, proximal mucosas, and colorectal tumors. BRCA1 expression was not significantly elevated in the tumor group compared to the normal mucosa group and was not associated with tumor DNA ploidy (Table I), nor was it correlated with TP53 or TERT expression (Table II). BRCA1 overexpression (>12% positivity) was measured in 22 out of 53 (41%) evaluated tumors, and was correlated with gain of chromosomal arm 17q21 where the *BRCA1* gene is localized (r=0.319, p=0.02). Eleven out of the 55 (20%) tumors had 17q21 gain and 91% of these were aneuploid tumors (p=0.04). Both TP53 and TERT levels were significantly elevated in the tumor group compared to normal mucosas, and TP53 expression (but not TERT expression) was significantly associated with DNA aneuploidy (p=0.01, Table I). TP53 expression showed no correlation with TERT expression (Table II). High TP53 levels (>23% positivity) were measured in 27 out of 55 (49%) tumors. TERT overexpression (>16% positivity) was measured in 26 out of 53 (49%) evaluated tumors, but was not correlated with gain of chromosomal arm 5p15.33 where the *TERT* gene which codes for telomerase is localized. Seven out of the 55 (13%) tumors had 5p15.33 gain and all were aneuploid tumors (p=0.03). Individually, BRCA1, TP53 and TERT expressions were not significantly

correlated with S-phase fraction (assessed by flow cytometric cell cycle analyses), Dukes' stage, tumor localization, nor with overall patient survival (data not shown).

Correlations of BRCA1, TP53 and TERT levels with AURKA, AURKB, MAD2 and BUB1B levels in the tumor group are shown in Table II. BRCA1 expression demonstrated strong correlations with the individual expressions of all four spindle proteins but was most strongly correlated with Ki-67 levels. BRCA1 expression was not correlated with any spindle protein, nor with Ki-67 in the normal mucosa group (Table III). TP53 expression was not correlated with the expression of any spindle protein, nor with Ki-67 in either the tumor or normal mucosa groups (Tables II, III). TERT expression in the tumor group showed a moderate correlation with MAD2L1 and a trend towards correlation with AURKA expression, but no correlations with any other spindle protein or with Ki-67 (Table II). In the normal mucosa group, TERT was significantly individually correlated to AURKA, AURKB, MAD2L1 and Ki-67 proteins (Table III).

Discussion

The BRCA1 tumor levels were not associated with aneuploidy and were similar to the BRCA1 levels measured for normal mucosa. The findings that 59% of the tumors examined had reduced BRCA1 levels (<12% positivity) was fairly consistent with previously published data for loss of heterozygosity (LOH) at the *BRCA1* locus, which has been reported to range from 40% (28) to 49% (28, 29) in colorectal carcinomas generally. BRCA1 overexpression (in the remaining 41% of the tumors) was correlated with gain of chromosomal arm 17q21 where the *BRCA1* gene is localized, but was not associated with tumor aneuploidy. Aneuploidy was however clearly correlated with 17q21 gain, indicating that genes other than *BRCA1* at this chromosomal locus may play a role in the aneuploidization process, a scenario which will be investigated using array comparative genomic hybridization (CGH) and fluorescence *in situ* hybridization (FISH). The BRCA1 protein detected in the present study was most likely wild-type, since *BRCA1* gene mutations are known to be infrequent in sporadic colorectal cancer (13). BRCA1 was not significantly correlated with TP53, perhaps not unexpectedly since the high frequency of *TP53* mutations (and resultant mutant proteins) that characterize the majority of sporadic colorectal carcinomas might be expected to affect *BRCA1* transcription since *BRCA1* is a TP53 target gene. Thus it could be expected that BRCA1 is non-functional due to *TP53* loss-of-function mutations in the majority of sporadic colorectal tumors (predominantly DNA aneuploid/CIN phenotype). An interesting question is whether BRCA1 is inactivated in colorectal tumors with a wild-type *TP53* genotype (predominantly DNA diploid/microsatellite instability (MIN) phenotype)), and if so, how. A recent study showed that *BRCA1* mutations appeared to be more frequently detected in replication error (RER)-positive (MIN phenotype) sporadic endometrial tumors compared to RER-negative (CIN phenotype) tumors (30), but the situation remains unclear for sporadic colorectal cancer. It would also be interesting to ascertain whether LOH at *BRCA1* is more prevalent in DNA diploid tumors, which presumably have functional TP53 compared to DNA aneuploid tumors, the majority of which have non-functional TP53. As LOH at the *BRCA1* locus was not assessed in the present work, one can only speculate as to how BRCA1 might be inactivated. BRCA1 was significantly correlated to each of the mitotic spindle proteins examined in the present work in the tumor group but these correlations were absent in the normal mucosa group, suggesting that overexpression of mitotic spindle proteins and BRCA1 inactivation (overexpression) occur together. Several interesting questions include: what impact does overexpression of the individual mitotic spindle proteins have on BRCA1 functionality and does mitotic spindle protein overexpression in and of itself compromise spindle protein function generally in sporadic colorectal cancer? Functional studies are needed to answer these questions and are currently in the planning stage. Loss of AURKA *via* RNA interference-mediated knockdown was recently shown to result in reduced phosphorylation of BRCA1 and compromised BRCA1 function (25), since AURKA normally binds to and phosphorylates BRCA1 during the G_2M transition, but this was not relevant for the present study as overexpression of AURKA was detected in the present tumor material (22).

We and others have previously demonstrated an association between TP53 expression and colorectal tumor aneuploidy (20, 21) and this association was re-confirmed in the present study. The order of events remains unclear, *i.e.* whether *TP53* mutations precede aneuploidy or *vice versa*. Our recent study of genetic aberrations in sorted diploid and aneuploid fractions from DNA aneuploid colorectal tumors suggested that *TP53* deletions do not precede large-scale aneuploidization in colorectal cancer (31), but where *TP53* mutations fit into the sequence of events in the progression to gross aneuploidy remains unclear. Since functional TP53 plays an important role in DNA damage response, DNA repair and apoptosis induction in response to apoptotic stimuli, inactivation of TP53 would be expected to contribute to overall genomic instability since tumor cells with abnormal genomes would survive and flourish in the absence of functional TP53. We and others have previously shown that the acquisition of a (mutant) TP53 phenotype is associated with lower spontaneous apoptosis and higher expression of the apoptotic inhibitor B-cell CLL/lymphoma 2 (BCL2) in sporadic colorectal cancer, suggesting that apoptosis is

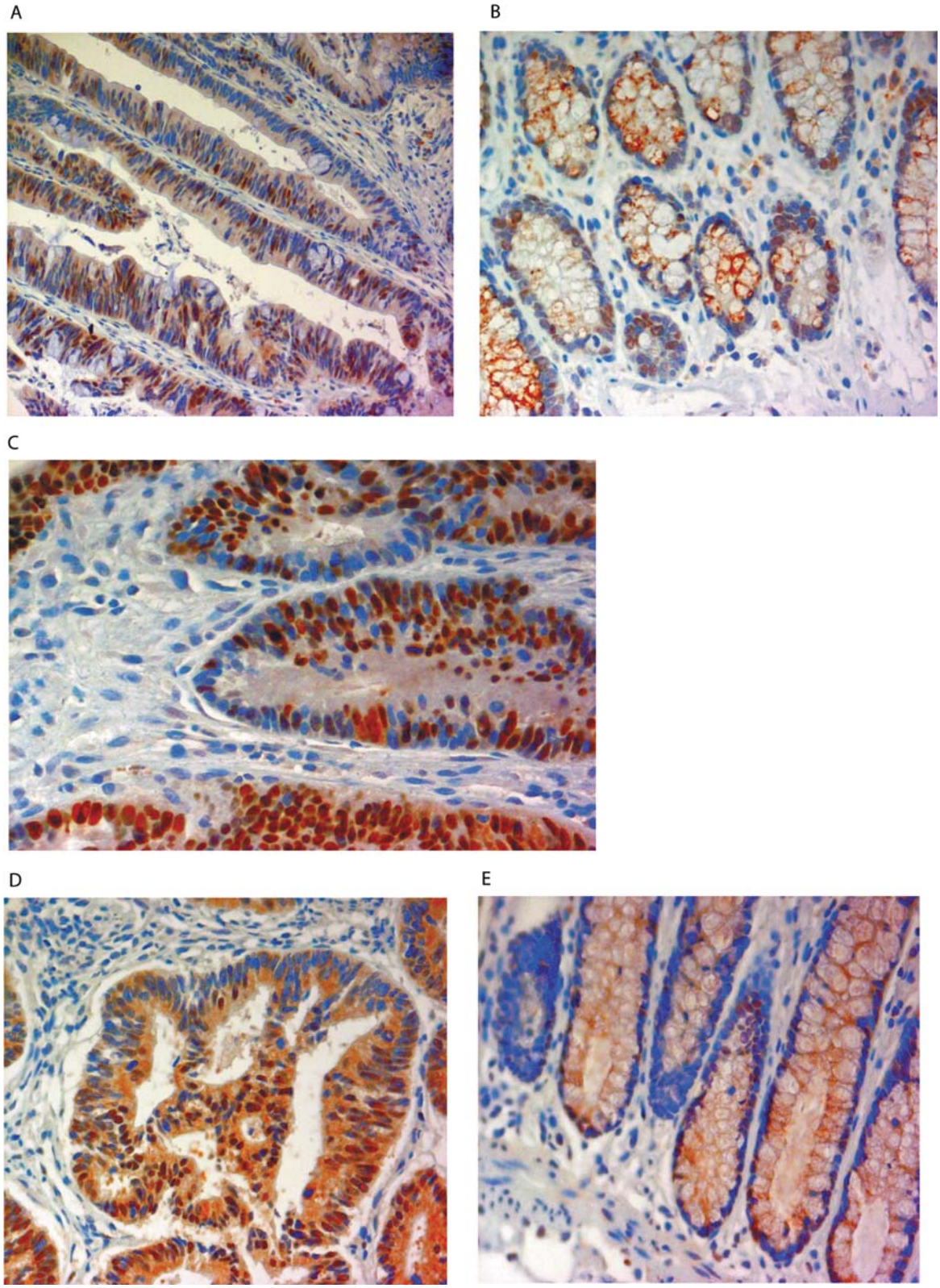

Figure 1. *Representative BRCA1, TP53 and TERT staining in colorectal tumor tissue and normal mucosa. A) BRCA1 staining in tumor tissue; B) BRCA1 staining in normal mucosa; C) TP53 staining in tumor tissue (normal mucosa was negative for TP53); D) TERT staining in tumor tissue, and E) TERT staining in normal mucosa. Original magnification, ×480.*

Table I. *Expression levels of BRCA1, TP53 and TERT in colorectal tumors and normal mucosa and associations with DNA ploidy.*

Parameter	% Positivity: normal mucosa (n=17)	% Positivity: peritumoral mucosa (n=20)	% Positivity: tumors (n=55)	*p*-Value	% Positivity: aneuploid tumors	% Positivity: diploid tumors	*p*-Value
BRCA1	9 (±3)	9 (±5)	12 (±7)	0.37	12 (±7)	12 (±7)	1.00
TP53	0 (range 0-4)	0 (range 0-3)	23 (range 0-91)	<0.0001	40 (0-85)	11 (4-91)	0.01
TERT	3 (range 4-13)	0 (range 0-58)	16 (range 0-83)	<0.0001	16 (0-83)	15 (0-70)	0.77

Table II. *Correlations of BRCA1, TP53 and TERT expressions with each other and with AURKA, AURKB, MAD2L1 and BUB1B expressions in 55 colorectal tumors.*

Parameter	Correlated with	r-Value	*p*-Palue
BRCA1 levels	TP53 levels	0.218	0.116
	TERT levels	0.239	0.092
	AURKA levels	0.474	0.000
	MAD2L1 levels	0.358	0.009
	AURKB levels	0.432	0.001
	BUB1B levels	0.407	0.003
	Ki-67 levels	0.497	0.000
TP53 levels	TERT levels	0.032	0.819
	AURKA levels	–0.066	0.636
	MAD2L1 levels	0.105	0.448
	AURKB levels	0.071	0.604
	BUB1B levels	–0.133	0.334
	Ki-67 levels	–0.032	0.817
TERT levels	AURKA levels	0.268	0.055
	MAD2L1 levels	0.273	0.048
	AURKB levels	0.040	0.778
	BUB1B levels	0.029	0.839
	Ki-67 levels	0.116	0.407

Table III. *Correlations of BRCA1, TP53 and TERT expressions with each other and with AURKA, AURKB, MAD2L1 and BUB1B expressions in 37 normal mucosas.*

Parameter	Correlated with	r-Value	*p*-Value
BRCA1 levels	TP53 levels	0.056	0.799
	TERT levels	0.054	0.803
	AURKA levels	0.128	0.552
	MAD2L1 levels	0.280	0.185
	AURKB levels	0.232	0.276
	BUB1B levels	0.087	0.701
	Ki-67 levels	0.077	0.722
TP53 levels	TERT levels	0.219	0.199
	AURKA levels	0.115	0.509
	MAD2L1 levels	0.226	0.186
	AURKB levels	0.284	0.093
	BUB1B levels	0.264	0.132
	Ki-67 levels	0.260	0.125
TERT levels	AURKA levels	0.365	0.029
	MAD2L1 levels	0.445	0.006
	AURKB levels	0.462	0.004
	BUB1B levels	0.242	0.168
	Ki-67 levels	0.482	0.003

deregulated following loss of TP53 function (32, 33). We have also previously shown that a high number of chromosomal aberrations were associated with a mutated *TP53* genotype, suggesting that *TP53* mutation and CIN may be linked (5). As was the case for BRCA1, an interesting question is whether overexpression of any of the mitotic spindle proteins contributes to TP53 inactivation in sporadic colorectal cancer. AURKA overexpression leading to (wild-type) TP53 degradation and down-regulation of checkpoint-response pathways (24) would not be expected to be a significant mechanism of TP53 inactivation in sporadic colorectal cancer, since the majority of tumors have *TP53* mutations with resultant accumulation/ stabilization of mutant TP53 proteins, which is the major mechanism for inactivation of *TP53*. This is supported by the present data showing lack of correlation of AURKA and TP53 (predominantly mutant) expressions. However, AURKA overexpression could be a contributory factor to TP53 inactivation in colorectal tumors without *TP53* mutations. However, as *TP53* mutation analyses were not performed on this tumor material, this cannot be determined, nor can it be concluded with certainty that high levels of TP53 reflect gene mutation in all cases, since they could in some few cases also reflect activation of wild-type TP53 which is involved in the response to genomic stress/DNA damage. In any case, since most of the TP53 proteins detected in the present tumor group are likely to be mutant, AURKA overexpression would have little effect on the degradation of mutant TP53 proteins since they have altered conformations and longer half-lives compared to wild-type TP53. AURKA has also been shown to phosphorylate TP53 at Ser215 leading to abrogation of its DNA binding and transactivation activity (23). Downstream TP53 target genes such as cyclin-dependent kinase inhibitor 1A (*CDKN1A*) and phosphatase and tensin homolog (*PTEN*) were shown to be

inhibited by AURKA in a Ser215 phosphorylation-dependent manner (23). Phosphorylation of *TP53* at this residue by AURKA might thus be an important mechanism of inactivating TP53, especially in colorectal tumors that have not otherwise undergone *TP53* mutation, and assessment of the phosphorylation status of TP53 at this residue is planned.

Uncapped telomeres resulting from telomere shortening trigger CIN (and aneuploidy) (19) through breakage-fusion-bridge cycles. Such genomic damage leads to cell cycle checkpoint activation and cell cycle arrest in normal cells that do not overexpress TERT (telomerase), whereas most cancer cells overexpress TERT and may also have defective DNA damage checkpoint functions, for example due to inactivation of TP53 or retinoblastoma pathways, such that they may not recognize breakage-fusion-bridge damage as DNA damage or are unable to induce apoptosis. TERT expression was not associated with colorectal tumor aneuploidy, consistent with the overall consensus that TERT expression constitutes a general tumor phenotype, nor was TERT overexpression (in *circa* 50% of the tumors) correlated with gain of chromosomal arm 5p15.33 where the *TERT* gene is localized. However, gain of 5p15.33 was associated with tumor aneuploidy, suggesting that genes other than *TERT* at this chromosomal locus are likely to play a role in the aneuploidization process, as was suggested for 17q21 gain and *BRCA1*. TERT was recently shown to be transcriptionally regulated by BRCA1, suggesting that BRCA1 is involved in telomere maintenance (34). While a trend towards a weak correlation between TERT and BRCA1 in the tumor group was detected, it did not reach the level of significance; however, this lack of significance does not necessarily imply that the correlation is not biologically relevant. A trend towards a correlation of TERT expression with AURKA expression was seen in the present tumor series, which may be consistent with the reported induction of TERT by AURKA overexpression (26). TERT expression was significantly correlated with expression of spindle checkpoint protein MAD2L1, suggesting that overexpression of this protein may be involved in the regulation of telomerase activity. It has previously been shown that TERT deficiency led to disruption of functional meiotic spindles and misalignment of chromosomes during meiotic division of murine oocytes (35), supporting putative interactions of TERT with one or more mitotic spindle proteins. Intriguingly, the significant correlations of TERT with AURKA and AURKB in the normal mucosa group were lost in the tumor group, suggesting that the acquisition of TERT overexpression in tumors is not involved in and does not affect the regulation of these mitotic spindle proteins.

In conclusion, BRCA1 inactivation is more likely to be the result of *TP53* mutation and resultant loss of *TP53* function and less the result of overexpression of any particular mitotic spindle protein, despite its strong correlations with all of the spindle proteins examined in the present work. The loss of *TP53* function is likely to be a key factor in the emergence of genomic instability since it is permissive for the formation and survival of aberrant (deregulated) colonic mucosal cells.

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References

1 Kops GJPL, Weaver BAA and Cleveland DW: On the road to cancer: aneuploidy and the mitotic checkpoint. Nat Rev Cancer *5*: 773-785, 2005.

2 Ried T, Knutzen R, Steinbeck R, Blegen H, Schröck E, Heselmeyer K, du Manoir S and Auer G: Comparative genomic hybridization reveals a specific pattern of chromosomal gains and losses during the genesis of colorectal tumors. Genes Chromosomes Cancer *15*: 234-245, 1996.

3 Lengauer C, Kinzler KW and Vogelstein B: Genetic instability in colorectal cancers. Nature *386*: 623-627, 1997.

4 Cahill DP, Lengauer C, Yu J, Riggins GJ, Willson JK, Markowitz SD, Kinzler KW and Vogelstein B: Mutations of mitotic checkpoint genes in human cancers. Nature *392*: 300-303, 1998.

5 De Angelis PM, Clausen OPF, Schjølberg A and Stokke T: Chromosomal gains and losses in primary colorectal carcinomas detected by CGH and their associations with tumour DNA ploidy, genotypes and phenotypes. Br J Cancer *80*: 526-535, 1999.

6 Zhou H, Kuang J, Zhong L, Kuo WL, Gray JW, Sahin A, Brinkley BR and Sen S: Tumour-amplified kinase STK15/BTAK induces centrosome amplification, aneuploidy and transformation. Nat Genet *20*: 189-193, 1998.

7 Baker DJ, Jeganathan KB, Cameron JD, Thompson M, Juneja S, Kopecka A, Kumar R, Jenkins RB, de Groen PC, Roche P and van Deursen JM: BubR1 insufficiency causes early onset of aging-associated phenotypes and infertility in mice. Nat Genet *36*: 744-749, 2004.

8 Hanks S, Coleman K, Reid S, Plaja A, Firth H, Fitzpatrick D, Kidd A, Méhes K, Nash R, Robin N, Shannon N, Tolmie J, Swansbury J, Irrthum A, Douglas J and Rahman N: Constitutional aneuploidy and cancer predisposition caused by biallelic mutations in *BUB1B*. Nat Genet *36*: 1159-1161, 2004.

9 Michel LS, Liberal V, Chatterjee A, Kirchwegger R, Pasche B, Gerald W, Dobles M, Sorger PK, Murty VV and Benezra R: MAD2 haplo-insufficiency causes premature anaphase and chromosome instability in mammalian cells. Nature *409*: 355-359, 2001.

10 Nguyen HG, Chinnappan D, Urano T and Ravid K: Mechanism of aurora-B degradation and its dependency on intact KEN and A-Boxes: identification of an aneuploidy-promoting property. Mol Cell Biol *25*: 4977-4992, 2005.

11 Ota T, Suto S, Katayama H, Han ZB, Suzuki F, Maeda M, Tanino M, Terada Y and Tatsuka M: Increased mitotic phosphorylation of histone H3 attributable to AIM-1/aurora-B overexpression contributes to chromosome number instability. Cancer Res *62*: 5168-5177, 2002.

12 Rajagopalan H and Lengauer C: Aneuploidy and cancer. Nature *432*: 338-341, 2004.

13 Wang Z, Cummins JM, Shen D, Cahill DP, Jallepalli PV, Wang TL, Parsons DW, Traverso G, Awad M, Silliman N, Ptak J, Szabo S, Willson JK, Markowitz SD, Goldberg ML, Karess R, Kinzler KW, Vogelstein B, Velculescu VE and Lengauer C: Three classes of genes mutated in colorectal cancers with chromosomal instability. Cancer Res *64*: 2998-3001, 2004.
14 Weaver Z, Montagna C, Xu X, Howard T, Gadina M, Brodie SG, Deng CX and Ried T: Mammary tumors in mice conditionally mutant for Brca1 exhibit gross genomic instability and centrosome amplification yet display a recurring distribution of genomic imbalances that is similar to human breast cancer. Oncogene *21*: 5097-5107, 2002.
15 Xu X, Weaver Z, Linke SP, Li C, Gotay J, Wang XW, Harris CC, Ried T and Deng CX: Centrosome amplification and a defective G_2-M cell cycle checkpoint induce genetic instability in *BRCA1* exon 11 isoform-deficient cells. Mol Cell *3*: 389-395, 1999.
16 Pihan GA and Doxsey SJ: The mitotic machinery as a source of genetic instability in cancer. Semin Cancer Biol *9*: 289-302, 1999.
17 Feldser DM, Hackett JA and Greider CW: Telomere dysfunction and the initiation of genome instability. Nat Rev Cancer *3*: 623-627, 2003.
18 Gollin SM: Mechanisms leading to chromosomal instability. Semin Cancer Biol *15*: 33-42, 2005.
19 Cheung AL and Deng W: Telomere dysfunction, genome instability and cancer. Front Biosci *13*: 2075-2090, 2008.
20 Meling GI, Lothe RA, Borresen AL, Graue C, Hauge S, Clausen OP and Rognum TO: The *TP53* tumour suppressor gene in colorectal carcinomas. II. Relation to DNA ploidy pattern and clinicopathological variables. Br J Cancer *67*: 93-98, 1993.
21 De Angelis PM, Stokke T, Smedshammer L, Lothe RA, Meling GI, Rofstad M, Chen Y and Clausen OPF: p53 expression is associated with a high degree of tumor DNA aneuploidy and incidence of *p53* gene mutation, and is localized to the aneuploid component in colorectal carcinomas. Int J Oncol *3*: 305-312, 1993.
22 Burum-Auensen E, DeAngelis PM, Schjolberg AR, Roislien J, Mjaland O and Clausen OP: Reduced level of the spindle checkpoint protein BUB1B is associated with aneuploidy in colorectal cancers. Cell Prolif *41*: 645-659, 2008.
23 Liu Q, Kaneko S, Yang L, Feldman RI, Nicosia SV, Chen J and Cheng JQ: Aurora-A abrogation of p53 DNA binding and transactivation activity by phosphorylation of serine 215. J Biol Chem *279*: 52175-52182, 2004.
24 Katayama H, Sasai K, Kawai H, Yuan ZM, Bondaruk J, Suzuki F, Fujii S, Arlinghaus RB, Czerniak BA and Sen S: Phosphorylation by aurora kinase A induces Mdm2-mediated destabilization and inhibition of p53. Nat Genet *36*: 55-62, 2004.
25 Ouchi M, Fujiuchi N, Sasai K, Katayama H, Minamishima YA, Ongusaha PP, Deng C, Sen S, Lee SW and Ouchi T: BRCA1 phosphorylation by aurora-A in the regulation of G_2 to M transition. J Biol Chem *279*: 19643-19648, 2004.
26 Yang H, Ou CC, Feldman RI, Nicosia SV, Kruk PA and Cheng JQ: Aurora-A kinase regulates telomerase activity through c-Myc in human ovarian and breast epithelial cells. Cancer Res *64*: 463-467, 2004.
27 Burum-Auensen E, De Angelis PM, Schjølberg AR, Kravik KL, Aure M and Clausen OP: Subcellular localization of the spindle proteins aurora A, Mad2, and BUBR1 assessed by immunohistochemistry. J Histochem Cytochem *55*: 477-486, 2007.
28 Garcia JM, Rodriguez R, Dominguez G, Silva JM, Provencio M, Silva J, Colmenarejo A, Millan I, Muñoz C, Salas C, Coca S, España P and Bonilla F: Prognostic significance of the allelic loss of the *BRCA1* gene in colorectal cancer. Gut *52*: 1756-1763, 2003.
29 Garcia-Patino E, Gomendio B, Lleonart M, Silva JM, Garcia JM, Provencio M, Cubedo R, España P, Ramón y Cajal S and Bonilla F: Loss of heterozygosity in the region including the *BRCA1* gene on 17q in colon cancer. Cancer Genet Cytogenet *104*: 119-123, 1998.
30 Koul A, Nilbert M and Borg A: A somatic *BRCA2* mutation in RER^+ endometrial carcinomas that specifically deletes the amino-terminal transactivation domain. Genes Chromosomes Cancer *24*: 207-212, 1999.
31 De Angelis PM, Stokke T, Beigi M, Flatberg G, Enger M, Haug K, Aass HC, Schjølberg A, Andresen PA, Ariansen S, Bø AS, Mjåland O and Clausen OP: Chromosomal 20q gain in the DNA diploid component of aneuploid colorectal carcinomas. Int J Cancer *120*: 2734-2738, 2007.
32 Sinicrope FA, Roddey G, McDonnell TJ, Shen Y, Cleary KR and Stephens LC: Increased apoptosis accompanies neoplastic development in the human colorectum. Clin Cancer Res *2*: 1999-2006, 1996.
33 De Angelis PM, Stokke T, Thorstensen L, Lothe RA and Clausen OP: Apoptosis and expression of Bax, Bcl-x, and Bcl-2 apoptotic regulatory proteins in colorectal carcinomas, and association with p53 genotype/phenotype. Mol Pathol *51*: 254-261, 1998.
34 French JD, Dunn J, Smart CE, Manning N and Brown MA: Disruption of BRCA1 function results in telomere lengthening and increased anaphase bridge formation in immortalized cell lines. Genes Chromosomes Cancer *45*: 277-289, 2006.
35 Liu L, Blasco MA and Keefe DL: Requirement of functional telomeres for metaphase chromosome alignments and integrity of meiotic spindles. EMBO Rep *3*: 230-234, 2002.

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