Evidence That BRCA1- or BRCA2-Associated Cancers Are Not Inevitable

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Inheriting a BRCA1 or BRCA2 gene mutation can cause a deficiency in repairing complex DNA damage. This step leads to genomic instability and probably contributes to an inherited predisposition to breast and ovarian cancer. Complex DNA damage has been viewed as an integral part of DNA replication before cell division. It causes temporary replication blocks, replication fork collapse, chromosome breaks and sister chromatid exchanges (SCEs). Chemical modification of DNA may also occur spontaneously as a byproduct of normal processes. Pathways containing BRCA1 and BRCA2 gene products are essential to repair spontaneous complex DNA damage or to carry out SCEs if repair is not possible. This scenario creates a theoretical limit that effectively means there are spontaneous BRCA1/2-associated cancers that cannot be prevented or delayed. However, much evidence for high rates of spontaneous DNA mutation is based on measuring SCEs by using bromodeoxyuridine (BrdU). Here we find that the routine use of BrdU has probably led to overestimating spontaneous DNA damage and SCEs because BrdU is itself a mutagen. Evidence based on spontaneous chromosome abnormalities and epidemiologic data indicates strong effects from exogenous mutagens and does not support the inevitability of cancer in all BRCA1/2 mutation carriers. We therefore remove a theoretical argument that has limited efforts to develop chemoprevention strategies to delay or prevent cancers in BRCA1/2 mutation carriers.

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INTRODUCTION
Inheriting a BRCA1 or BRCA2 gene mutation can cause a dual deficiency both in repairing some complex DNA damage and in forming sister chromatid exchanges (SCEs) for damage that cannot be repaired. Thus, any source of ongoing complex DNA damage places demands for normal BRCA1/2 gene products. This scenario creates a theoretical limit that effectively means BRCA1/2-associated cancers occur spontaneously and cannot be prevented or delayed.

Some DNA lesions have been viewed as an integral part of DNA replication before cell division (1), and they cause temporary replication blocks and replication fork collapse. This kind of replication stress can particularly affect genomic loci such as fragile sites, telomeres and repetitive sequences (2). DNA integrity is especially threatened if spontaneous reactions occur during the process of copying DNA before mitosis. During DNA copying, DNA polymerases stall at noncoding sites of DNA damage, and the replication fork may collapse. Stalled and collapsed replication forks can lead to chromosome breaks and micronucleus formation. They can also trigger SCEs (3). SCEs are widely believed to be a cytological manifestation of the repair of damaged or collapsed replication forks that occur all the time during DNA replication (4).

At the molecular level, SCE and homologous recombination are very similar. Identical (SCE) or similar (homologous recombination) segments of DNA line up and then cross over. SCEs do not necessarily lead to adverse health outcomes, but rather indicate complicated DNA damage that can be produced by many mutagens.

The persistence of lesions involved in SCEs for several cell divisions implies that, if lesions are not eliminated, SCEs represent a mechanism to tolerate them, allowing damaged cells to more safely replicate. The lesions may eventually be eliminated by repairs that take place during or after replication. SCEs and chromosomal breaks often occur in the same chromosomal regions (5). A high rate of spontaneous SCEs, even in normal individuals, has been used as major evidence for DNA damage during replication. Cancers would then become an almost inevitable consequence of cell division if BRCA1/2-mediated pathways are crippled.

However, much of the evidence for a high rate of spontaneous SCEs and DNA
mutation is based on measurements made with bromodeoxyuridine (BrdU). BrdU is incorporated into newly synthesized DNA in dividing cells instead of deoxythymidine. Differential staining of BrdU-modified DNA allows the newly synthesized chromatid to be recognized because BrdU incorporation causes much weaker staining (6). When pieces of a BrdU chromatid exchange with its sister (which does not have BrdU), SCEs are directly visualized and can be counted.

SCE assays only measure sister chromatid crossover events, but do not measure noncrossover recombination. The frequency of noncrossover events is not necessarily tethered to that of crossover events. Thus, a low SCE rate is not necessarily sufficient to establish low mutagenic potential. However, only four mutations were found by direct sequencing of the human Y chromosome after 13 generations, and these were only single base substitutions (7). This produces an estimate of about 100 mutations over all human chromosomes per generation. Moreover, human DNA has remained stable because the human genome still shares >96% homology with chimpanzees, despite an accelerated mutation rate of the Y chromosome and the 5–7 million years since divergence (8,9). The estimate from direct Y-chromosome sequencing is consistent with estimates from published human chimpanzee comparisons for the same Y-chromosomal region and with other estimates of the rates of human mutation. Mutations that would alter the structure of some proteins such as fibrinopeptides occur once or twice in an estimated 200,000 years.

Other Repairs Are Normal or Alternatives Exist

Reactive oxygen and nitrogen species are spontaneous mutagens that can affect DNA. In the 1990s, the use of inappropriate methods led to an overestimate of DNA oxidation products by up to three orders of magnitude (10). There are dedicated enzymes to repair some of this damage. For example, a DNA glycosylase prevents mutations associated with 8-oxoguanine, a common product of oxidative DNA damage (11), and this enzyme participates in base excision repair. 8-Oxoguanine is usually repaired before DNA replication and may not contribute to carcinogenesis. Other repair pathways such as nonhomologous end joining are still normal and are unaffected by the BRCA1/2 mutation. In BRCA1/2 mutation carriers, response even to some potent mutagens is still normal (12,13). Alternative less precise repairs may suffice, even if a normal BRCA1/2-mediated recombination pathway is crippled. Cells that have not lost the recombination process may have a growth advantage over cells with biallelic mutations. This step minimizes the effect of BRCA1/2 deficits in permitting spontaneous mutations.

Other protective mechanisms are still normal. These mechanisms include checkpoint pathways and sterile protection because new DNA is rapidly assembled onto nucleosomes, where it is supercoiled and tightly wound. After entry into mitosis, some fraction of chromosome regions with errors in DNA replication becomes sequestered into nuclear compartments containing p53 binding protein 1. These 53BP1 nuclear bodies shield DNA regions with replication problems against erosion and transmit them to future cell generations (2).

Finally, the whole organism has safeguards missing from cell culture. These include an intact immune system, detoxification systems and hazard avoidance. Although Brca1 null strains can be generated in the mouse, homozygous loss of BRCA1 is incompatible with human life. In these artificial mouse strains, mammary tumors appear to be spontaneous, but even low doses of ionizing radiation cause a marked increase in tumor formation (14). The mice also have increased tumors within the digestive tract on exposure to oxidative carcinogens. Thus, the homozygous mouse Brca1 mutation increases risks from carcinogenic exposures. This raises the question whether some seemingly spontaneous tumors are actually related to ionizing radiation or other environmentally caused carcinogens and might therefore be preventable.

Study Rationale

The present study was designed to test the data underlying the idea that cancer risk in BRCA1/2 mutation carriers is so tightly linked to DNA replication that every time cells in BRCA1/2 mutation carriers divide, they accumulate chromosome aberrations. If this is true, exogenous genotoxins are unnecessary, and cancer becomes an unavoidable consequence of DNA replication (15).

Here, evidence is summarized that shows that the extent of spontaneous DNA damage manifested by SCEs has probably been overestimated. This finding brings SCE measurements closer to other measures of spontaneous DNA damage in mutation carriers. Measurement of DNA mutation by direct DNA sequencing of multiple generations suggests that non–SCE-associated mutation events are rare as well. Finding that SCEs have been overestimated reduces the numbers of BRCA1/2–associated cancers that are predicted as an inevitable consequence of cell replication. In contrast, the response to exogenous mutagens and carcinogens is clearly affected by mutation within pathways that require BRCA1 and BRCA2. This evidence removes a theoretical argument that has limited efforts to develop chemoprevention strategies to delay or prevent cancers in BRCA1/2 mutation carriers. There may be safe and feasible methods to control or compensate for DNA damage that triggers chromosome damage or chromatid exchanges in nonmutation carriers. Data supporting chemoprevention and other interventions in BRCA1/2 mutation carriers may already exist but should still be considered as hypothetical. Considerably more investigation is needed before adopting patient care recommendations.

MATERIALS AND METHODS

Homologous recombination often uses the nascent sister chromatid to repair potentially lethal DNA lesions accompany-
ing replication (3). BRCA1/2 genes encode for proteins required for repairs that suppress some SCEs, and BRCA1/2 proteins are also required for these crossover events (16). Numbers of SCEs in the absence of added mutagens were therefore used to measure the overall requirements for BRCA1/2 proteins caused by endogenous DNA damage. Searches for “BRCA1 and SCE” produced only five references. Searches were expanded to include ATM or Fanconi proteins and SCE. This is justified because ATM and Fanconi gene (FA) products participate with BRCA1/2 proteins in a common pathway. PubMed literature references for all available years were searched for the words “sister chromatid exchanges” with the addition of words such as “in vivo,” “nutrition,” “diet,” “spontaneous” and “antioxidant.” The literature was scanned for effects of protectants and dietary intervention on the rates of SCEs. Key words included “SCE induction,” “SCE diet,” “SCE reduction” and “SCE chemicals.” Because SCEs commonly occur at fragile sites, similar searches were conducted through the fragile site literature. Many studies made reference to chromosomal aberrations and micronuclei, and these specific terms were used in additional searches.

**Statistical Analysis**

Testing of mean values representing varying numbers of samples was done by calculating two-sample t values (17,18) for comparisons of means from unpaired data. This assumes continuous distributions not too far from bell-shaped curves. Comparisons of mean values in the presence of mutagen versus control values were conducted by dozens of multiple two-sample t tests. Statistical significance data in the original article were included for articles reporting protection against SCE accompanying mutagen exposure. SCE control numbers versus SCEs in the presence of mutagens in studies of one to three individuals were compared by two-sample t testing of weighted averages and by the summary method of metaanalysis. Statistical calculations were done by using the StatsDirect statistical program and with Microsoft Excel. The summary method of metaanalysis was done with StatsDirect.

**RESULTS**

To test whether BRCA1/2-related cancers are an unavoidable consequence of cell replication, numbers of spontaneous SCEs were compiled from the literature to estimate the normal rate of ongoing DNA recombination. Reports in Table 1 all used BrdU to measure background SCE. The first eight rows in Table 1 are references that reported mean values for spontaneous SCEs. Comparing these eight mean values by two-sample t tests (17) showed that most of the values have statistically significant differences from each other. There are 28 possible two-sample t test comparisons of spontaneous SCE levels in the eight references. Of these 28 two-sample t test comparisons, 24 showed statistically significant differences, no matter if the variances were assumed to be equal or unequal (two-tailed P < 0.05, with most <0.0001, all at high power).

Very large increases in SCEs versus controls can occur in the presence of exogenous mutagens (Table 1). Mutagens listed in the first five rows of Table 1 give greater numbers of SCEs than the spontaneous SCE values in the same row, and the differences are statistically significant (Table 1, last column). In contrast, the differences in SCEs associated with smoking versus controls (Table 1) are not statistically significant, with low power to detect differences. However, Table 1 does not consider differences in numbers of cells with high frequencies of SCEs. Data in Table 5 (see below) also refer to smoking, and Shim et al. (19) did find a statistically significant difference (P < 0.01) with high power to detect differences.

### Table 1. Rates of spontaneous SCE determined by BrdU in normal human lymphocyte cultures differ among laboratories but increase greatly in response to exogenous mutagens.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Spontaneous SCEs ± SD per cell determined by BrdU; n</th>
<th>Test mutagen</th>
<th>SCEs ± SD (max)/cell with mutagen; n</th>
<th>Two-sample unpaired t test results, mutagen SCEs versus spontaneous SCEs</th>
</tr>
</thead>
<tbody>
<tr>
<td>43</td>
<td>7.01 ± 1.24; 22</td>
<td>Chemotherapy</td>
<td>11.52 ± 3.33; 24</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>44</td>
<td>5.175 ± 0.607; 31</td>
<td>Chlorimethane hydrochloridea</td>
<td>18.433 ± 4.523; 10</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>45</td>
<td>8.4 ± 0.11; 5</td>
<td>Ptaquiloside</td>
<td>26.1 ± 0.14; 5</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>46</td>
<td>2.54 ± 0.82; 20</td>
<td>Type 1 diabetes</td>
<td>5.44 ± 1.47; 35</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>47</td>
<td>21.84 ± 4.21; 20</td>
<td>White fluorescent light in S phase</td>
<td>28.37 ± 5.98</td>
<td>P = 0.0003</td>
</tr>
<tr>
<td>48</td>
<td>6.9 ± 0.9; 22</td>
<td>Smoking</td>
<td>8.4 ± 1.2; 18</td>
<td>NS</td>
</tr>
<tr>
<td>49</td>
<td>7.16 ± 1.13; 15</td>
<td>Smoking</td>
<td>8.65 ± 1.43; 14</td>
<td>NS</td>
</tr>
<tr>
<td>50</td>
<td>9.32 ± 1.0; 8</td>
<td>Smoking</td>
<td>10.76 ± 1.36; 6</td>
<td>NS</td>
</tr>
<tr>
<td>51</td>
<td>4.70 ± 0.24; 2</td>
<td>MMCb</td>
<td>34.82 ± 1.25; 2</td>
<td>P &lt; 0.0001 for weighted averages of SCEs from MMC versus SCEs in control cells (51–53)</td>
</tr>
<tr>
<td>52</td>
<td>4.45 ± 0.68; 4</td>
<td>MMCb</td>
<td>53.37 ± 5.1; 4</td>
<td></td>
</tr>
<tr>
<td>53</td>
<td>5.95 ± 0.31; 1</td>
<td>MMC</td>
<td>25.95 ± 0.81; 1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.40 ± 0.59; 1</td>
<td></td>
<td>23.52 ± 1.30; 1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.64 ± 0.47; 1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. Number of subjects; NS, not statistically significant.
b. MMC greatly increased the frequency of chromosomal aberrations as independent supporting evidence.
Mitomycin C (MMC) is a mutagen and DNA cross-linking agent that requires pathways containing BRCA1/2 and Fanconi proteins for repairs. In counting SCEs by BrdU methods, MMC causes large increases (Table 1, last three rows). Doses of MMC that cause between 33% and 90% reduction in relative growth, increase SCEs about 100-fold (20), provided the cells remain viable. The last three rows in Table 1 (references 51–53) were based on measurements from one to four individuals. Summary metaanalysis of these isolated data points gives a combined value of 5.39 (confidence interval [CI] 4.68–6.19) with an inconsistency statistic of 95.2% (highly inconsistent). Meta-analysis of MMC-associated SCEs gave a much higher summary value of 32.53 (25.34–41.75) but again with a very high inconsistency value (99.2%). There is no overlap in the CIs for the two groups, supporting the idea that the difference is statistically significant.

To further explore the role of chance in explaining differences attributed to MMC, the weighted arithmetic mean of the spontaneous SCE values (Table 1, bottom three rows) was calculated as 5.30 ± 0.15. The weighted mean of the number of SCEs in the presence of MMC was calculated as 29.51 ± 0.58. This increase in SCEs due to the presence of MMC was statistically significant at a high power (>99%). A two-sample t test of spontaneous versus MMC weighted average values for SCEs gave

\[ P < 0.0001, \]

whether or not variances were assumed to be equal.

### Table 2. Spontaneous SCEs measured in different systems or by different methods differ greatly from SCEs measured in human lymphocytes.

<table>
<thead>
<tr>
<th>System and reference</th>
<th>Assay</th>
<th>Spontaneous SCE frequency</th>
<th>Test mutagen</th>
<th>SCE (max)/cell with mutagen</th>
<th>Independent supporting data</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHO cells in culture (54)</td>
<td>BrdU extrapolated to low doses</td>
<td>1.32/cell/cell cycle</td>
<td>Lower SCE frequency in vivo</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bovine autosomes 16 and 26 and Y chromosomes (55)</td>
<td>BrdU for one replication then CO-FISH</td>
<td>3.7/metaphase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ear fibroblasts from FYDR mice (20)</td>
<td>Fluorescent phenotype generated by homologous recombination event on chromosome 1</td>
<td>1.2–1.4 recombinants per million cell divisions</td>
<td>MMC</td>
<td>−150 recombinant cells/million cell divisions</td>
<td></td>
</tr>
<tr>
<td>FYDR mice carrying a transgene; spontaneous HR in pancreas that contains few S phase cells (23)</td>
<td>Fluorescent phenotype representing homologous recombination event</td>
<td>2.5 recombinant cells/million cell divisions; some mice have zero recombinants; −10/million cells in pancreas of individual animals (range ~0–300)</td>
<td>MMC</td>
<td>−90–135 per million cell divisions</td>
<td></td>
</tr>
<tr>
<td>Intact mouse embryo (21)</td>
<td>Expression of acetylcholine receptor due to recombination</td>
<td>1–2 × 10⁻⁶/cell division</td>
<td>Frequency of recombination in intact embryo is similar to that in cultured cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human fibroblasts from a Lesch-Nyhan donor (56)</td>
<td>Growth in HAT medium due to recombination at HPRT locus</td>
<td>10–30 × 10⁻⁶ per cell per generation</td>
<td>Methyl nitrosoguanidine, UV, chromium oxide, MMC, DES, nickel chloride, sodium azide</td>
<td>Dose-dependent increase</td>
<td>Noncarcinogens showed much less activity Dose-dependent induction of HAT reversion is concordant with in vivo carcinogenesis</td>
</tr>
</tbody>
</table>

CHO, Chinese hamster ovary; CO-FISH, chromosome orientation fluorescence in situ hybridization; FYDR, fluorescent yellow direct repeat; HR, homologous recombination; HAT, hypoxanthine, amethopterin (methotrexate) and thymidine; UV, ultraviolet irradiation; HPRT, hypoxanthine-guanine phosphoribosyltransferase; DES, diethylstilbestrol.
Most studies cited in Table 1 provided independent evidence supporting the idea that exogenous mutagens (rather than chance) are associated with increased SCE levels. This evidence includes comet assay results that are independent of BrdU, dose-response relationships, greatly increased chromosome aberrations, concordance with carcinogenicity under limited specialized conditions and the absence of SCEs with nonmutagens.

In Table 2, extrapolation of BrdU concentrations to low doses shows just over one SCE per cell division (Table 2, first row). In vivo methods generally give lower rates of SCE than in vitro methods (Table 2). In contrast to using BrdU to measure overall spontaneous recombination, specialized methods that mark a specific DNA region give results that are millions of times less than the low dose value of one SCE per cell division and the values in Table 1. These results yield about one or only a few recombinants per million cell divisions. By use of an artificial gene transferred to mice that fluoresces yellow on recombination, recombination rates of about 1 per million cell divisions were obtained. Spontaneous recombination rates of between 1 and $2 \times 10^{-6}$ were also measured using tandem repeat sequences (21). Many juvenile mice have zero recombinants, and there is a wide range of recombinant cell frequencies (22) (Table 2).

Spontaneous recombination levels of 15.6 ($\pm$ 1.6) per million cells are threefold lower than the background levels observed for immortalized human cells at the same duplication (23). Either value is far below widely cited rates of spontaneous recombination. Background SCEs and homologous recombination are lower in normal cells, which undergo homologous recombination much less frequently than immortal cell lines.

There is only limited data related to SCEs in BRCA1/2 mutation carriers, but some studies have measured chromosome damage or SCEs in human cells with defects in other genes related to BRCA1/2 functions. Fanconi anemia is a rare syndrome characterized by bone marrow failure, malformations and cancer predisposition. In Fanconi anemia,
one of the genes encoding a Fanconi protein has an inherited homozygous or biallelic mutation. Both the genomic deficit and the cancer risks are more serious than a heterozygous BRCA1/2 mutation. Fanconi anemia has long been considered a spontaneous chromosome fragility syndrome. Leukemia is common but the age of onset of leukemia is variable (24). On average, the Fanconi anemia population has an increased level of spontaneous DNA damage, but 54% of patients with Fanconi anemia (excluding mosaics) have a spontaneous chromosome fragility level within the range of normal patients (25). Therefore, spontaneous chromosome fragility cannot be used as a diagnostic tool for Fanconi anemia. For Fanconi anemia G, background levels of chromatid interchanges and other aberrations in Fanconi anemia are also close to those of normal cells (26) (Table 3).

<table>
<thead>
<tr>
<th>Reference</th>
<th>Number of patients mutation-negative</th>
<th>Relationship to mutation carrier</th>
<th>Length of follow-up</th>
<th>Breast and other cancer risk (CI) and comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>33</td>
<td>1,492</td>
<td>FDR</td>
<td>Mean 6.1 years, followed up to age 75</td>
<td>Standardized incidence ratio (SIR) 4.3 Ovarian and colon cancer risks were slightly below normal</td>
</tr>
<tr>
<td>60</td>
<td>104</td>
<td>FDR/SDR</td>
<td>Median 8 years</td>
<td>SIR 2.9 (1.0–8.6)</td>
</tr>
<tr>
<td>61</td>
<td>11,378</td>
<td>FDR</td>
<td>NA</td>
<td>Relative risk (RR) 0.39 (0.04–3.8); substantial risk heterogeneity High-risk women account for only 3.4% of the female populations in three countries studied, but 32% of all breast cancer cases; uses a constant value for risk of carriers; assumes one additional latent gene to account for all risk</td>
</tr>
<tr>
<td>62</td>
<td>184</td>
<td>FDR</td>
<td>Entry at 31.3 years followed for average of 17.7 years to age 48; third-degree relative to age 41</td>
<td>SIR 5.0</td>
</tr>
<tr>
<td>63</td>
<td>395</td>
<td>FDR/SDR/TDR</td>
<td>For FDR, SIR 1.33 (0.49–2.91); all RR = 0.75 (0.34–1.41); assumptions about oophorectomy</td>
<td></td>
</tr>
<tr>
<td>64</td>
<td>130.5</td>
<td>FDR</td>
<td>Diagnosis before age 50</td>
<td>Odds ratio (OR) 2</td>
</tr>
<tr>
<td>65</td>
<td>442</td>
<td>FDR/SDR</td>
<td>Median 6.1 years</td>
<td>SIR 1.29 (0.58–2.88); excluded 400 women with a prior cancer diagnosis</td>
</tr>
<tr>
<td>66</td>
<td>375</td>
<td>FDR/SDR mostly</td>
<td>Median age 44 mean follow up 4.9 years</td>
<td>SIR 2.3 (0.57–9.19) for in situ breast cancer</td>
</tr>
<tr>
<td>67</td>
<td>3,742</td>
<td>FDR</td>
<td>NA</td>
<td>OR 1.6 (1.2–2.1)</td>
</tr>
</tbody>
</table>

FDR/SDR/TDR, first-/second-/third-degree relatives; NA, not specified.
rations in Fanconi cell lines can still show significant overlap with normal cells (25). Two Fanconi lymphoblastoid cell lines had only about one to two times the number of spontaneous chromatid breaks as the wild-type cell line.

Fibroblast cell lines derived from Fanconi anemia type A patients show about two times greater spontaneous chromatid

### Table 5. Common mutagens that increase SCE and protective factors that reduce SCEs measured with BrdU.

<table>
<thead>
<tr>
<th>SCE control/cell</th>
<th>System tested with BrdU and reference</th>
<th>Test protective agent</th>
<th>Mutagen and SCE with mutagen</th>
<th>SCE with protectant</th>
<th>Statistical test of significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonsmokers</td>
<td>Blood specimens from clinically healthy male subjects between 20 and 52 years of age (19)</td>
<td>Green tea and plant polyphenols</td>
<td>Cigarette smokers, 9.46 ± 0.46</td>
<td>Smoker SCEs (7.94 ± 0.31) were comparable to those of nonsmokers (7.03 ± 0.33)</td>
<td>Smoking cigarettes and drinking green tea significantly affected SCE frequency and explained 32.7% of SCE variation (P &lt; 0.002) (19)</td>
</tr>
<tr>
<td></td>
<td>7.03 ± 0.33 (n = 9); smokers (9.46 ± 0.46) (n = 14)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.36 ± 0.24</td>
<td>Mouse bone marrow cells in vivo (68)</td>
<td>Green tea extract given to anesthetized animals</td>
<td>Dimethyl-nitrosamine 23.40 ± 0.94 at 18 h</td>
<td>12.12 ± 0.07 (a similar effect at 24 h; more modest effects at other times)</td>
<td>The suppression rate was significantly different from mice given only dimethyl nitrosamine (t test P &lt; 0.05) (68)</td>
</tr>
<tr>
<td>4.1 ± 0.46 for not fasted (29% cells have 0–2 SCEs)</td>
<td>Male Wistar rats every other day fasting 12 wks (69)</td>
<td>Dietary limitations</td>
<td>Diet</td>
<td>1.8 ± 0.12 for fasted (72% cells have 0–2 SCEs)</td>
<td>Significant differences in the numbers of SCEs (t test P &lt; 0.05) (69)</td>
</tr>
<tr>
<td>4 at 6 h to 6 at 48 h exposure to radiation</td>
<td>Human peripheral blood lymphocytes exposed to 1.8 Ghz radiation (70)</td>
<td>Ginkgo biloba</td>
<td>RF/microwave radiation 8 at 6 h to 13 at 48 h</td>
<td>6 at 6 h and about 7 at 48 h</td>
<td>There was a significant increase (P &lt; 0.05) in SCE frequency in RF-exposed lymphocytes compared with sham controls (70)</td>
</tr>
<tr>
<td>0.24 ± 0.12/cell</td>
<td>Traffic policemen in Bangkok Thailand (71)</td>
<td></td>
<td>4.40 ± 0.93/cell with Benzene, toluene, CO, formaldehyde, etc.</td>
<td>A significantly higher SCE frequency in policemen was observed (P &lt; 0.05) (71)</td>
<td></td>
</tr>
<tr>
<td>4.3 ± 0.19</td>
<td>Human lymphocytes (72)</td>
<td></td>
<td>6.64 ± 0.88 with hydroquinone, a product of benzene metabolism</td>
<td>Hydroquinone significantly increased micronuclei and SCE (P &lt; 0.0001) (72)</td>
<td></td>
</tr>
<tr>
<td>4.6 ± 0.37 to 6.0 ± 0.39/cell</td>
<td>CHO-K1 cells (73)</td>
<td>Ascorbate, glutathione</td>
<td>8.6 ± 0.52 Phenyl hydroquinone, a metabolite of o-phenylphenol, an agricultural fungicide and surface disinfectant (Lysol)</td>
<td>Ascorbate and glutathione significantly decrease SCEs versus Phenyl hydroquinone alone (P &lt; 0.05) (73)</td>
<td></td>
</tr>
<tr>
<td>12/cell</td>
<td>Ataxia telangiectasia patient cells (affects BRCA1/2-mediated pathways) (4)</td>
<td></td>
<td>18 after 2 Gy radiation</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Continued
aberrations. However there are higher levels in other cell lines (25,27) (Table 3).

**Evidence That Spontaneous Rates of DNA Damage Are Low in Mutation Carriers**

Data in Table 2 suggest there are actually only small numbers of spontaneous SCEs. Table 3 shows that chromatid aberrations and micronuclei are often near normal, even for severe deficits. BRCA1 and BRCA2 carriers with or without breast cancer do not exhibit a marked difference in chromosome stability relative to controls (28,29). However, damage from MMC is greatly amplified in mutation carriers to the point of cell death. About half the in vivo and in vitro studies find a mutation-associated increase in chromosome damage from radiation (30). Recent epidemiologic data provide further evidence that even low-level radiation doses (such as from chest X-rays) increase cancer risks in BRCA1/2 mutation carriers (31,32).

Data in Table 3 therefore further implicate environmental factors or other gene variants in increasing cancer incidence in BRCA1/2 families. To test this idea, we next determined whether such modifiers have been detected in epidemiological studies. Table 4 presents cancer risks in members of BRCA families who did not have the familial BRCA1 mutation. Although most studies in Table 4 suggest an increase in risk in BRCA1/2 family members who do not have the familial mutation, confidence intervals often include one. One study did find statistically significant breast cancer risk was higher for women between the ages of 50 and 70 years (1% per year) than for women between the ages of 30 and 50 years (0.4% per year) (33). Yet other studies in Table 4 did not follow family members past age 50 years. Close relatives of patients with breast cancer are more likely than other women to belong to a high-risk breast cancer group, even if they do not have the familial BRCA1 mutation. Inconsistency in results among the studies suggests additional variables. These could include environmental differences and variation in other genes, which may also be sensitive to environment or diet. A large population-based, case-control study (34) found broad variation in breast cancer risk among carriers of BRCA1 and BRCA2 mutations. There was no single risk associated with having a BRCA1/2 mutation.

**Healthy Carrier and Noncarriers in BRCA Families Show About the Same Amounts of DNA Damage**

Changing the environment to one with lower cancer rates may lower the incidence of breast cancer in mutation carriers. Ashkenazi Jewish women are approximately 10 times more likely to be
**BRCA1/2** mutation carriers than other women; therefore, Ashkenazi Jewish women have high breast cancer risks as a group. In contrast, the breast cancer risk of the Brazilian population is much lower (roughly half the rate of the U.S. population). Ashkenazi Jewish women who move to Sao Paulo or Porto Alegre, Brazil, reduce their breast cancer mortality much closer to the lower breast cancer mortality of the general Brazilian population at all ages except age >79 years. Adjusted breast cancer mortality rates were 24.1/100,000 (CI 13.5–39.7) among Ashkenazi women in the two cities in Brazil and 22.3/100,000 women in the general population. These data support the influence of the environment on breast cancer risk. Even a high-risk population can reduce their cancer risks (35).

**Chemoprotection.** Despite its overestimation of spontaneous endogenous DNA damage and its other potential limitations, BrdU has been used to compare effectiveness of protection against mutation-related exogenous damage. The effects of these or other mutagens such as viruses cannot be fully explained by increased oxidative stress. Mutagens listed in Table 5 include known carcinogens, and exposure to them can probably be reduced or avoided. Green tea, dietary limitation, ascorbate, glutathione, broccoli and correction of niacin deficiency have been suggested as capable of preventing the increase in SCE levels (Table 5). Most articles in Table 5 verified the statistical significance of their findings (Table 5, last column). Table 5 therefore shows that mutagens increase the numbers of SCE and that there may be specific pharmacologic agents that can prevent some of this increase.

**DISCUSSION**

We hope that this work will be a prelude to a much-needed discussion of the inevitability of hereditary cancers. There is a popular assumption that high levels of DNA cross-linking, double strand breaks and their resolution are going on all the time during cell replication. DNA is thought to be under further constant attack from byproducts of normal ongoing processes. BRCA1/2 mutation carriers have a dual deficit in repairing complex DNA damage and in forming SCEs. It follows that a mutation in **BRCA1** or in **BRCA2** genes means that cancers in mutation carriers are inevitable. Preventing cancers related to **BRCA1/2** deficiencies has not been viewed as realistic. The rate of normal spontaneous SCEs reflecting endogenous damage has been especially overestimated and depends on the method of measurement. A lowered rate of spontaneous SCEs agrees with low rates of spontaneous DNA mutation, direct DNA sequencing and measurements of spontaneous chromosome damage. Until recently, some oxidation products have also been overestimated, and experimental support for spontaneous oxidative cross-links is not strong. All these considerations reduce the requirements for **BRCA1/2**-mediated processes. Moreover, independent evidence shows that environmental and other genetic factors are also involved in determining risk. Risks are elevated for breast cancers in members of **BRCA1/2** families who do not have the familial mutation. Even mouse models with very severe **Brca1** deficits (14) show increased cancer risks from their environment. Changing the environment by moving to an area with lower cancer rates may reduce **BRCA** mutation–related breast cancer risks (35).

Another single gene differences from **BRCA1/2** deficiencies can be managed by pharmacology, by diet and/or by other interventions. Pharmacologic interventions in **BRCA1/2** mutation carriers have largely concentrated on better methods of destroying damaged cells or on blocking the estrogen receptor (36). The data in Table 5 imply that there are chemopreventive agents in foods or natural products that oppose the effects of some mutagens. Whether these interventions will reduce the cancer burden in **BRCA1/2** mutation carriers requires further testing.

Recent genomic data have reported that breast cancers are mutationally heterogeneous. There is substantial variation in somatic mutation and in indels. The ability to suppress mutagen-associated SCEs depends on **BRCA1/2** pathway activity. Genomic results are quite consistent with differential exposures and responses to mutagens as contributors to shaping cancer tissue distribution (37,38). Very little is known about what causes these mutations. Whether carcinogenicity of some mutagens is susceptible to chemoprevention requires further testing. Chemoprevention may be helpful whether the source is exogenous or endogenous.

A recent model on the basis of cancer DNA sequence results suggests clusters of DNA lesions precede large-scale genomic rearrangements. Sequences of **BRCA1**- and **BRCA2**-associated breast cancers show enrichment of somatic rearrangements and fusions in critical protein coding genes (39). Some mutagens such as radiation exposure and inflammation can encourage these kinds of rearrangements by causing extensive and complex DNA damage that collapses replication forks, causes double strand breaks or requires homologous recombination repairs. This increases requirements for **BRCA1/2** pathway activities, but the mutagens are exogenous.

Another source of mutation could be related to micronutrient deficits. Epidemiological studies reveal strong association between micronutrient deficiencies and development of cancer. A prevailing idea is that marginal micronutrient deficiencies lead to allocation of scarce cellular resources toward immediate survival at the expense of maintaining genomic integrity. This result places the individual at greater risk for degenerative diseases and cancer in old age (40).

BrdU-based methods have probably overestimated spontaneous SCEs because BrdU is itself a mutagen and a potent carcinogen that causes SCEs (41). DNA lesions caused by BrdU are difficult to avoid in replicating cells because BrdU enters the nucleotide pool and then becomes incorporated into DNA. BrdU induces transcription of silenced genes, disrupts nucleosome positioning by inducing A-form–like DNA conformation.
in yeast cells, induces a senescent type phenotype, decondenses particular regions of chromosomes and alters specific chromatin structures (42).

A limitation of our results lies in the extent to which BrdU amplifies the effects of other mutagens. Higher levels of BrdU incorporation may increase the sensitivity of detection of both spontaneous and mutagen-related SCEs. However, in normal cells, exogenous increases in SCEs measured with BrdU are consistent with other systems of measurement, such as fluorescence detection of recombination and comet assays (Table 2).

Dose response curves and extrapolation to low BrdU doses also support a large difference between SCEs caused by BrdU versus other mutagens.

CONCLUSION

Pathways containing BRCA1 and BRCA2 gene products are thought essential to repair spontaneous complex DNA damage or to carry out SCEs if repair is not possible. A high level of natural, unavoidable complex DNA damage represents a theoretical limit that would effectively mean most BRCA1/2-associated cancers are spontaneous and cannot be prevented or delayed.

Much evidence, however, for high rates of spontaneous DNA mutation is based on measuring SCEs using BrdU. Here we find that the routine use of BrdU has probably led to overestimating spontaneous DNA damage and SCEs because BrdU is itself a mutagen. Evidence based on spontaneous chromosome abnormalities and epidemiologic data indicate strong effects from exogenous mutagens and does not support the inevitability of cancer in all BRCA1/2 mutation carriers. We therefore remove a theoretical argument that has limited efforts to develop chemoprevention strategies to delay or prevent cancers in BRCA1/2 mutation carriers.

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DISCLOSURE

The authors declare that they have no competing interests as defined by Molecular Medicine, or other interests that might be perceived to influence the results and discussion reported in this paper.

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