Chromosomal damage in peripheral blood lymphocytes of newly diagnosed cancer patients and healthy controls

Pavel Vodicka1,2, Zdenka Polivkova2, Sylvie Sytara2, Hana Demova2, Marie Kucerova2, Ludmila Vodickova1,3, Veronika Polakova1,2, Alessio Naccarati1, Zdenek Smerhovsky3,4, Miloslav Ambruš5, Marie Cerna2 and Kari Hemminki6,7

1Department of molecular biology of cancer, Institute of Experimental Medicine, Academy of Science of Czech Republic, Videska 1083, 14200 Prague 4, Czech Republic, 2Department of General Biology and Genetics, 3rd Faculty of Medicine, Charles University, Prague, Czech Republic, 3Toxicogenomics Unit, National Institute of Public Health, Srobarova 48, 100 42 Prague, Czech Republic, 4Department of Epidemiology, 2nd Faculty of Medicine, Charles University, 150 00 Prague, Czech Republic, 5Department of Radiology and Oncology, Faculty Hospital Kralovske Vinohrady, 100 42 Prague, Czech Republic, 6Division of Molecular Genetic Epidemiology, German Cancer Research Center, Heidelberg 691 20, Germany and 7Center for Primary Health Care Research, Lund University, Malmö 205 02, Sweden

Email: pvodicka@biomed.cas.cz

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Background: The majority of human cancers arise from cells unable to maintain genomic stability. Recent prospective studies indicated that enhanced chromosomal aberrations (CAs) frequencies are predictive of gastrointestinal and lung cancers. However, studies on incident cancer patients are lacking; thus, we investigated chromosomal damage in newly diagnosed cancer patients and healthy individuals. Methods: We analyzed chromosomal damage in peripheral blood lymphocytes in a group of 300 incident cancer patients (with different malignancies) in comparison with 300 healthy controls. Results and Conclusions: The frequencies of aberrant cells (ACs) and CAs were significantly higher in patients (2.38 ± 1.56 and 2.53 ± 1.69, respectively) as compared with controls (1.81 ± 1.31 and 1.94 ± 1.47, respectively, P < 0.01). The percentual difference in chromatid-type aberrations (CTAs) between patients and controls was moderately significant (1.37 ± 1.20 and 1.11 ± 0.99, respectively, P ≤ 0.05), whereas the difference in chromosome-type aberrations (CSAs) was stronger (1.16 ± 1.24 versus 0.83 ± 1.12, P < 0.01). Using binomial logistic regression, the estimated odds ratios and 95% confidence interval for ACs were 1.33 (1.18–1.49), P < 0.01; for CAs, 1.27 (1.14–1.41), P < 0.01; for CTA, 1.24 (1.07–1.44), P < 0.01 and for CSA, 1.27 (1.10–1.47), P < 0.01. By stratifying patients for distinct neoplasia, markers of chromosomal damage were significantly enhanced in patients with breast, prostate and head/neck cancers, whereas no effect was recorded in patients affected by gastrointestinal cancers. The present study shows for the first time evidence of increased chromosomal damage in lymphocytes of incident cancer patients compared with healthy controls. The effects were observed in different cancer types but as the number of patients was relatively small, further studies are warranted.

Materials and methods

Cytogenetic analysis was carried out on 300 patients with newly diagnosed tumor disease (patients of the Department of Radiotherapy and Oncology, Faculty Hospital Kralovske Vinohrady, Prague, Czech Republic) and 300 controls of similar age, sex and socioeconomic background (recruited from the Blood Center of Faculty Hospital Kralovske Vinohrady, Prague, Czech Republic).

The patients consisted of 146 women and 154 men (mean age 62.8 ± 10.4 years), 157 individuals were non-smokers, 58 former smokers (who quit smoking ≥ 5 years ago) and 85 smokers. The control individuals exhibited a mean age of 56.7 ± 13.6 years; there were 109 women and 191 men. Smoking status was not influenced by sex, age or by the time between cytogenetic analysis and cancer detection. The strongest association was found for gastrointestinal cancers and, to a lesser extent, for lung cancer (15–18). However, no association was detected for matching elevated also in newly diagnosed cancer patients.

We conducted the first case–control study on CAs in peripheral blood lymphocytes of newly diagnosed cancer patients and healthy controls. The goal is to examine whether an increased frequency of CAs is directly associated with cancer. Such data would add evidence to the previous prospective studies that have been unable to exclude CAs as only exposure markers. Furthermore, several types of cancer are included assuming that the effects may differ.

Introduction

Existing data suggest that the majority of human cancers arise from cells unable to maintain genomic stability, often due to altered DNA repair mechanisms (1–3). Chromosomal aberrations (CAs), a validated marker of cancer susceptibility, are one of few available intermediately cytogenetic end points for human cancer. The principal lesions in the process of CAs formation are DNA double-strand breaks (4,5). The cytogenetic changes in peripheral lymphocytes, such as CAs, reflect human genotoxic exposure, but also the individual sensitivity toward genotoxins, and can be used as biomarkers of an early effect of genotoxic carcinogens (6–8). The frequencies of some cytogenetic biomarkers (particularly micronuclei) increase with age (9) and female gender (10), as this effect is very probably mediated by the frequent loss of one X chromosome. Tobacco smoking increases the level of CAs and sister chromatid exchanges (6,11).

In several epidemiologic prospective studies, an increased risk of cancers in individuals with high level of CAs in peripheral blood lymphocytes was detected, as summarized by Bonassi et al. (12–14) and Norppa et al. (15). These studies revealed that the CA frequency could be predictive of cancer risk apparently independent of exposure to carcinogens because the effect remained after stratification for exposure. The association between the level of CAs and the risk of cancer was not influenced by smoking, sex, age or by the time between cytogenetic analysis and cancer detection. The strongest association was found for gastrointestinal cancers and, to a lesser extent, for lung cancer (15–18). However, no association was detected for sister chromatid exchanges and micronuclei (19,20). Regarding the types of CAs, Hagmar et al. (21) found a similar pattern of predictivity of cancer for both chromosome-type aberrations (CSAs) and chromatid-type aberrations (CTAs). On the other hand, Rössner et al. (16) and Boffetta et al. (17) found significant association only for the CSAs, but not for CTAs, whereas Smerhovsky et al. (22) found the association of cancer risk with aberrant cells (ACs) and chromatid breaks but did not find any association for chromatin exchanges and CSAs in miners exposed to radon. However, the chain of evidence linking CAs to the risk of cancer would be stronger if it was shown that CAs are elevated also in newly diagnosed cancer patients.

The most frequent malignancies in the group of patients were breast (75 cases), colorectal (68 cases) and prostate (57 cases, Figure 1) cancers. For CAs analysis, the blood samples were taken from the patients before any treatment to avoid additional chromosomal changes. Heparinized blood samples were cultivated in a RPMI 1640 medium (Sigma, St Louis, MO, USA) with 20% fetal calf serum (ZVOS Hustopece a.s., Hustopece, Czech Republic) and phytohemagglutinin (GIBCO, Paisley, UK) for 50 h (23). Mitotic division was arrested by colchicine (Sigma) added for the last 2 h. After the cytogenetic procedure (treatment with hypotonic solution and reagent fixation), slides were stained by conventional Giemsa staining. One hundred metaphases with 46 ± 1 chromosomes were blindly scored from each sample by two independent scorers. The percentage of ACs, the percentage of...
CAs and the individual types of aberrations were detected, i.e. CTAs (chromatid breaks and chromatid exchanges) and CSAs (chromosome breaks—terminal or interstitial—i.e. double minutes, dicentrics, rings and abnormal chromosomes). Gaps were scored but not included among CAs and excluded from statistical evaluation. Data are expressed as mean frequencies ± SD. As dicentric and ring chromosomes may pose an indicator of the diagnostic X-ray exposure, their frequencies were compared in the cases and the control group.

Data were analyzed with SPSS 13.0 for Windows software (Chicago, IL). The mean differences in the cytogenetic end points of interest were tested with the non-parametric Mann–Whitney U-test. The effects of the cytogenetic end points on the risk of cancer were evaluated by means of binomial logistic regression. Crude (unadjusted) estimates of the odds ratios (cORs) and odds ratios adjusted for effects of potential confounders (age and smoking) are reported with their 95% confidence limits.

Besides the assessment of the risk of cancer in general, we used binary logistic regression to examine also the relationships between the frequencies of the evaluated cytogenetic end points and occurrence of cancer in specific sites.

Results

According to Table I, the percentage of ACs in the group of patients was significantly higher (mean ± SD 2.38 ± 1.56) than in controls (1.81 ± 1.31, P < 0.01). Analogous differences were observed also for CAs (2.53 ± 1.69 in cancer patients versus 1.94 ± 1.47 in controls, P < 0.01) and CSAs (1.16 ± 1.24 versus 0.83 ± 1.12, P < 0.01). The moderate difference in percentages of CTAs between cases (1.37 ± 1.20) and controls (1.11 ± 0.99), however, did also reach the level of statistical significance (P < 0.05). By expressing the differences in distributions of the cytogenetic end points between cases and controls in terms of cancer risk (Table I), we observed statistically significant associations of ACs (cOR = 1.33, 95% confidence interval (CI) = 1.18–1.49), CAs (cOR = 1.27, 95% CI = 1.14–1.41), CTAs (cOR = 1.24, 95% CI = 1.07–1.44) and CSAs (cOR = 1.27, 95% CI = 1.10–1.47) with cancer risk. Figure 1 illustrates the distribution of frequencies of ACs in incident cancer patients and in healthy controls. Investigated individuals may arbitrarily be stratified (by using the median value of % of ACs) into groups with a low, medium and high frequency of ACs.

We did not find any significant differences in markers of chromosomal damage between males and females (when patients and controls were pooled), but both female and male patients exhibited significantly higher frequencies of ACs, total CAs and CSAs than corresponding female and male controls (data not shown).

In order to exclude a possible effect of the diagnostic X-ray exposures on the level of cytogenetic end points in cases, we also compared the frequencies of dicentric and ring chromosomes. The values of these cytogenetic end points were similar in both the cases and the control group (0.30 ± 0.59 versus 0.26 ± 0.61, P = 0.13 for dicentric chromosomes and 0.02 ± 0.13 versus 0.02 ± 0.14, P = 0.76 for ring chromosomes, respectively).

The risks of cancer associated with the evaluated cytogenetic end points adjusted for potential confounding by age and smoking are given in Table II. After the adjustment, all measured markers of chromosomal damage were significant predictors of cancer risk; an increase in one unit (i.e. by 1%) of ACs, CAs, CTAs and CSAs may result in an elevated risk of cancer by 27, 22, 21 and 22%, respectively. Regarding the confounders, associations of age and smoking with cancer risk are shown in Table II.

The distributions of the evaluated cytogenetic end point in studied subgroups are displayed in Figure 1. The most frequent cancer observed in cases was breast cancer (n = 75), followed by colorectal (n = 68), prostate (n = 57), uterus and ovary (n = 19), head and neck (n = 16), bladder and kidney (n = 18), stomach, gallbladder and pancreatic (n = 14), lungs (n = 11), melanoma and skin (n = 11) and other (n = 11) cancers.

Furthermore, by using binary logistic regression, we examined the relationships between the frequencies of the evaluated cytogenetic end points and occurrence of cancer in specific sites; the results are shown in Table III. Frequencies of ACs and CAs were significantly higher in patients with breast, prostate, uterus/ovary, head and neck and bladder/kidney cancers. CSAs frequency was significantly associated with occurrence of six types of cancer (breast, prostate, lungs, uterus/ovary, head and neck), thus representing better predictor of cancer risk than CTAs (Table III). Interestingly, no association between the frequencies of the evaluated cytogenetic end points and occurrence of gastrointestinal cancer (colorectum and stomach/gallbladder/pancreas) was found. By using the adjusted odds ratio for controlling confounders in the three most represented cancer types (Figure 2), breast cancer risk was associated predominantly with chromosomal damage, prostate cancer risk with chromosomal damage and age, whereas colorectal

### Table I. Chromosomal damage distribution in cases and controls and the estimate of cancer risk

<table>
<thead>
<tr>
<th>Chromosomal damage (%)</th>
<th>Controls (n = 300)</th>
<th>Cases (n = 300)</th>
<th>cOR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACs</td>
<td>1.81 ± 1.31</td>
<td>2.38 ± 1.56**</td>
<td>1.33</td>
<td>1.18–1.49</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CAs</td>
<td>1.94 ± 1.47</td>
<td>2.53 ± 1.69**</td>
<td>1.27</td>
<td>1.14–1.41</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CTAs</td>
<td>1.11 ± 0.99</td>
<td>1.37 ± 1.20*</td>
<td>1.24</td>
<td>1.07–1.44</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CSA</td>
<td>0.83 ± 1.12</td>
<td>1.16 ± 1.24**</td>
<td>1.27</td>
<td>1.10–1.47</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Statistically significant differences between patients and controls; the values were not adjusted for other confounders (cOR); significant values are in bold. *P ≤ 0.05, **P ≤ 0.01.

### Table II. Binary logistic regression models to discern the modulation of incident cancer by chromosomal damage end points and major confounders (such as age, sex and smoking)

<table>
<thead>
<tr>
<th>Incident cancer cases (n = 300) compared with controls (n = 300)</th>
<th>aOR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACs (%)</td>
<td>1.27</td>
<td>1.13–1.43</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Age (years)</td>
<td>1.04</td>
<td>1.02–1.05</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Smoking (1 for smokers)</td>
<td>1.32</td>
<td>0.93–1.86</td>
<td>0.12</td>
</tr>
<tr>
<td>CAs (%)</td>
<td>1.22</td>
<td>1.10–1.37</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Age (years)</td>
<td>1.04</td>
<td>1.02–1.05</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Smoking (1 for smokers)</td>
<td>1.33</td>
<td>0.94–1.87</td>
<td>0.11</td>
</tr>
<tr>
<td>CTAs (%)</td>
<td>1.21</td>
<td>1.03–1.41</td>
<td>0.02</td>
</tr>
<tr>
<td>Age (years)</td>
<td>1.04</td>
<td>1.02–1.05</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Smoking (1 for smokers)</td>
<td>1.40</td>
<td>1.00–1.97</td>
<td>0.05</td>
</tr>
<tr>
<td>CSAs (%)</td>
<td>1.22</td>
<td>1.05–1.40</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Age (years)</td>
<td>1.04</td>
<td>1.02–1.05</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Smoking (1 for smokers)</td>
<td>1.44</td>
<td>1.03–2.02</td>
<td>0.04</td>
</tr>
</tbody>
</table>

aOR (odds ratio adjusted for main confounders); significant values are in bold.
Table III. Odds ratios of the cytogenetic parameters for individual cancer types for each cytogenetic parameter, in comparison with 300 healthy controls

<table>
<thead>
<tr>
<th>Cancer</th>
<th>No.</th>
<th>ACs</th>
<th>OR</th>
<th>95% CI</th>
<th>P value</th>
<th>CAs</th>
<th>OR</th>
<th>95% CI</th>
<th>P value</th>
<th>CTAs</th>
<th>OR</th>
<th>95% CI</th>
<th>P value</th>
<th>CSAs</th>
<th>OR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>74</td>
<td>1.38</td>
<td>1.15–1.66</td>
<td>&lt;0.01</td>
<td>1.28</td>
<td>1.09–1.51</td>
<td>&lt;0.01</td>
<td>1.33</td>
<td>1.06–1.68</td>
<td>0.02</td>
<td>1.22</td>
<td>1.00–1.52</td>
<td>0.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colorectal</td>
<td>68</td>
<td>1.07</td>
<td>0.88–1.31</td>
<td>0.47</td>
<td>1.05</td>
<td>0.88–1.26</td>
<td>0.50</td>
<td>1.19</td>
<td>0.93–1.53</td>
<td>0.18</td>
<td>0.96</td>
<td>0.75–1.22</td>
<td>0.72</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prostate</td>
<td>57</td>
<td>1.36</td>
<td>1.11–1.67</td>
<td>&lt;0.01</td>
<td>1.30</td>
<td>1.09–1.55</td>
<td>&lt;0.01</td>
<td>1.25</td>
<td>0.96–1.62</td>
<td>0.11</td>
<td>1.28</td>
<td>1.03–1.58</td>
<td>0.02</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Lungs</td>
<td>11</td>
<td>1.33</td>
<td>0.88–2.02</td>
<td>0.18</td>
<td>1.20</td>
<td>0.82–1.74</td>
<td>0.35</td>
<td>0.71</td>
<td>0.31–1.42</td>
<td>0.34</td>
<td>1.47</td>
<td>1.00–2.17</td>
<td>0.05</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Uterus + ovary</td>
<td>19</td>
<td>1.45</td>
<td>1.05–2.01</td>
<td>&lt;0.01</td>
<td>1.35</td>
<td>1.02–1.80</td>
<td>0.04</td>
<td>1.16</td>
<td>0.74–1.83</td>
<td>0.51</td>
<td>1.40</td>
<td>1.02–1.92</td>
<td>0.04</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Head + neck</td>
<td>16</td>
<td>2.49</td>
<td>1.71–3.63</td>
<td>&lt;0.01</td>
<td>2.07</td>
<td>1.51–2.82</td>
<td>&lt;0.01</td>
<td>2.23</td>
<td>1.46–3.40</td>
<td>&lt;0.01</td>
<td>1.66</td>
<td>1.20–2.29</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Skin</td>
<td>11</td>
<td>1.44</td>
<td>0.96–2.17</td>
<td>0.08</td>
<td>1.51</td>
<td>1.08–2.13</td>
<td>0.02</td>
<td>0.89</td>
<td>0.47–1.69</td>
<td>0.71</td>
<td>1.76</td>
<td>1.24–2.51</td>
<td>&lt;0.01</td>
<td></td>
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</tr>
<tr>
<td>Stomach + gallbladder</td>
<td>14</td>
<td>1.11</td>
<td>0.75–1.63</td>
<td>0.61</td>
<td>1.03</td>
<td>0.72–1.47</td>
<td>0.89</td>
<td>0.96</td>
<td>0.56–1.65</td>
<td>0.89</td>
<td>1.08</td>
<td>0.68–1.69</td>
<td>0.75</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bladder + kidney</td>
<td>18</td>
<td>1.51</td>
<td>1.11–2.04</td>
<td>&lt;0.01</td>
<td>1.41</td>
<td>1.08–1.85</td>
<td>0.01</td>
<td>1.62</td>
<td>1.01–2.31</td>
<td>0.05</td>
<td>1.31</td>
<td>0.94–1.83</td>
<td>0.12</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>12</td>
<td>1.06</td>
<td>1.06–1.63</td>
<td>0.79</td>
<td>1.21</td>
<td>0.85–1.72</td>
<td>0.28</td>
<td>1.14</td>
<td>0.66–1.96</td>
<td>0.68</td>
<td>1.22</td>
<td>0.82–1.82</td>
<td>0.33</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Significant values are in bold; OR, odds ratio.

Fig. 2. The frequencies of chromosomal damage (ACs, CAs, CTAs and CSAs) according to the type of malignancies in comparison with the healthy control group. The data represent mean ± SD, the differences between the cancer patients and the healthy controls were evaluated by Mann–Whitney U-test; asterisks represent significant difference at P < 0.05, double asterisks represent significant difference at P < 0.01 and triple asterisks represent significant difference at P < 0.001. The numbers of patients for individual cancer are given in Results.

Discussion

It is generally accepted that majority of human cancers are related to genomic instability, including chromosomal instability. Alterations in the number of chromosomes are consistently observed in virtually all cancers. The fundamental question, whether they are the causes or rather consequences of malignant transformation, still remains open (25–27). Both structural and numerical CAs have been found to be rather consequences of malignant transformation, still remains open. The fundamental question, whether they are the causes or rather consequences of malignant transformation, still remains open.

In our study, the markers of chromosomal damage (ACs, CAs and CSAs) were significantly associated with the onset of cancer. In the present study, binomial regression models disclosed simultaneous modulation of cancer risk by chromosomal damage and by main confounders (such as age and smoking, known for long to modulate cancer risk). We describe for the first time that analyzed markers of chromosomal damage increase the cancer risk by 20–30%, whereas the associations of the onset of cancer with age and smoking were quantitatively less pronounced.

In our study, the markers of chromosomal damage (ACs, CAs and CSAs) were significantly elevated mainly in patients with breast, prostate, uterus/ovary and head/neck cancers in comparison with healthy controls, whereas they were almost identical in patients with gastrointestinal (colorectal and stomach/gallbladder/pancreas) cancers. CSAs were more strongly associated with the risk of incident cancer than CTAs. This is in accordance with previous prospective studies (16,17). An association of chromosomal damage with breast, prostate and head/neck cancers, but not with gastrointestinal and, partially, lung cancer (as reported in references 16–18) may be explained by the different character of the studies (a case–control one versus a prospective one) and by the limited number of patients with other cancers than breast, colorectal and prostate, which account for two-thirds of all incident cases. The lack of association between chromosomal damage and colorectal cancer is further documented by the adjusted ORs for controlling confounders, where colorectal cancer risk was mainly modulated by age and smoking. A relatively small difference in CAs, CTAs and CSAs between the patients and the controls (resembling the differences found in healthy individuals exposed to xenobiotics in the tire plant (36) may be due to the fact that the chromosomal damage has been assayed for in the surrogate...
(peripheral blood lymphocytes), whereas more dramatic numerical as well structural chromosomal changes could be expected in the target tissues.

Our study is the first report comparing CAs in incident (newly diagnosed) cancer patients and healthy controls. Our findings add to the existing data that the frequency of CAs in peripheral blood lymphocytes may be an early predictive marker of cancer risk. The study shows interesting differences in chromosomal damage among patients with tumors at various anatomic sites, which merits further investigation.

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Conflict of Interest Statement: None declared.

References

Chromosomal damage in incident cancer cases