DNA DAMAGE IN TUMOR CELLS AND PERIPHERAL BLOOD LYMPHOCYTES OF ENDOMETRIAL CANCER PATIENTS ASSESSED BY THE COMET ASSAY

L.G. Buchynska¹, O.V. Brieieva*, N.P. Iurchenko¹, V.V. Protsenko², S.V. Nespryadko²

¹R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, NAS of Ukraine, Kyiv 03022, Ukraine
²National Cancer Institute, MH of Ukraine, Kyiv 03022, Ukraine

To date, genome instability is considered to be a common feature not only of tumor cells, but also of non-malignant cells of cancer patients, including peripheral blood lymphocytes (PBLs). The issue of the association between genome instability in tumor cells and PBLs, as well as of its relationship with tumor progression remains poorly understood. Aim: To evaluate the level DNA damage in tumor cells and PBLs of endometrial cancer (EC) patients with regard to clinical and morphological characteristics of the patients. Materials and Methods: DNA damage was assessed in 106 PBLs samples and 42 samples of tumor cell suspension from EC patients by comet assay. PBLs from 30 healthy women were used as control. The level of DNA damage was expressed as the percentage of DNA in the comet tails (% tail DNA). Results: It was revealed that the amount of DNA damage in PBLs of EC patients was 2.2 times higher in comparison with that of healthy donors (8.3 ± 0.7 and 3.7 ± 0.4% tail DNA, respectively) (p < 0.05). In this study, no association between the levels of DNA damage in endometrial tumor cells and PBLs was observed (r = 0.11; p > 0.05). The amounts of DNA damage both in tumor cells and PBLs were not related to the degree of tumor differentiation as well as the depth of myometrial invasion, but depended on the body mass index (BMI) of EC patients: high level of lesions was observed in patients with elevated BMI values. Furthermore, the level of DNA damage in tumor cells was associated to familial aggregation of cancer and was significantly higher in endometrial cells from patients with family history of cancer vs that from EC patients with sporadic tumors (32.3 ± 2.9 and 22.8 ± 1.8% tail DNA, respectively) (p < 0.05). It was also found that for women who had high level of DNA damage in PBLs, the risk of EC was greater (odds ratio value of 3.5) compared to those with low level of such lesions. Conclusion: Genome instability that appears as an increased level of DNA damage in tumor cells and PBLs of EC patients is associated with BMI and family history of cancer and can reflect a predisposition to cancer.

Key Words: DNA damage, tumor cells, peripheral blood lymphocytes, endometrial cancer, comet assay.

Today, it is well known that the formation and progression of malignant neoplasms are accompanied by extensive molecular genetic changes. The presence of various types of DNA damage and chromosomal alterations is a characteristic feature of tumor cells [1]. According to modern notions, such changes are associated with genomic instability of malignant cells and reflect the influence of exo- and endogenous DNA-damaging factors, as well as defects in the functioning of the DNA repair systems [1, 2]. Genomic instability promotes the emergence of genetic diversity, clonal evolution of tumor cells and progression of the neoplastic process [3].

Modern studies indicate that endometrial carcinoma is characterized by the presence of microsatellite and chromosomal instability [4]. Moreover, endometrial cells are constantly exposed to the genotoxic influence of reactive oxygen species (ROS) resulting from metabolic transformations of estrogens during the menstrual cycle [5]. The elevated ROS level leads to the appearance of a highly mutagenic 8-oxo-7,8-dihydro-2′-deoxyguanosine, DNA breaks, apurin sites and chromosomal alterations [6]. Furthermore, genome instability of endometrial tumor cells may have hereditary origin, in particular due to germline mutations in the mismatch repair genes in the Lynch syndrome [7].

In recent years, numerous studies have been devoted to the problem of genome destabilization in malignant cells [8, 9]. However, today little is known about how the deregulation of genome integrity maintenance in carcinoma cells manifests at the systemic level, that is, on the structural and functional features of other cells in the body, including peripheral blood lymphocytes (PBLs). Meanwhile, in recent years, the issue of the possibility of using lymphocytes as surrogate markers (cells) that reflect the molecular genetic changes in the tumor is actively discussed [10–12].

A number of studies have shown that genome integrity in the PBLs of patients with different forms of cancer is disturbed [13–18]. Our previous study has revealed that PBLs of endometrial cancer (EC) are characterized by strong genome destabilization, in particular impaired DNA repair, which is associated with family history of cancer [21]. However, the issue of the relationship between genome instability in PBLs and tumor cells, as well as its association with tumor progression and clinical characteristics of patients with EC, remains insufficiently studied.

This study aims to analyze the association between DNA damage in PBLs and tumor cells of EC patients. In addition, the dependence of the degree of DNA damage on the clinical and morphological characteristics of patients and its significance to the risk of EC is studied.

MATERIALS AND METHODS

A total of 106 newly diagnosed, previously untreated patients with EC stages I and II were recruited...
Lymphocytes were isolated from venous blood by Ficoll-Hypaque gradient centrifugation method. To obtain single cell suspensions, tumor tissue was disrupted with a MEDI machine (Becton Dickinson). After disaggregation of the tissue, the cell suspension was filtered to remove any tissue debris. The viability of PBLs was determined using the trypan blue exclusion test.

In order to evaluate baseline levels of DNA damage in PBLs and tumor cells, single cell gel electrophoresis assay (Comet assay) was performed as described previously by Olive [22]. A suspension of lymphocytes or tumor cells (1 × 10^6 cells/ml) was mixed with 1% low melting point agarose (Sigma–Aldrich) at 37 °C, and 75 μl of this mixture was spread on slides precoated with 1% normal melting point agarose (Sigma–Aldrich). After solidification of the agarose, the slides were immersed into lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris base, 10% DMSO, 1% Triton X-100, pH 10). After disaggregation of the agarose, the slides were immersed into lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris base, 10% DMSO, 1% Triton X-100, pH 10). Lymphocytes treated with 100 μM H$_2$O$_2$ for 5 min. served as surrogate cells that reflect certain characteristics of malignant cells, a comparison analysis was performed between the levels of DNA damage in PBLs and endometrial tumor cells. In this study, no association between these parameters was observed (r = 0.11; p > 0.05).

The extent of the level of DNA damage in PBLs and tumor cells depends on the body mass index (BMI) of EC patients. Thus, individuals with BMI exceeding that in cells from patients with sporadic tumors (32.3 ± 2.9 and 22.8 ± 1.8% tail DNA, respectively) exceeded that in cells from patients with sporadic tumors (32.3 ± 2.9 and 22.8 ± 1.8% tail DNA, respectively).

The statistical analysis was performed using the Statistica 8.0 software package (StatSoft, Inc.). The Mann–Whitney U test was used to evaluate the differences between groups of EC patients and healthy women. A p-value less than 0.05 was considered statistically significant. Relationship between variables was determined using Spearman’s rank correlation coefficient (r). Logistic regression was used to calculate odds ratios (OR) and 95% confidence intervals (CI) for the association between DNA damage level in PBLs and EC risk.

**RESULTS**

Large inter-individual difference in the level of DNA damage in PBLs was observed in both healthy donors and EC patients. At the same time, the range of this parameter was wider in PBLs of EC patients (1.1–2.1% tail DNA) than that of control samples (0.2–0.6% tail DNA). On average, the level of DNA damage in PBLs of EC patients was 8.3 ± 0.7% tail DNA that was 2.2 times higher in comparison with its degree in healthy donors (3.7 ± 0.4% tail DNA) (p < 0.05). Analysis of the level of DNA damage in malignant cells was carried out on tumor tissue samples of 42 EC patients (7 ± well, 19 moderate and 16 poorly differentiated tumors). It was revealed that EC cells are characterized by a pronounced level of DNA damage. It was revealed 26.4 ± 1.8% tail DNA with individual variations ranging from 3.3 to 62.9% tail DNA (Fig. 1).

**Fig. 1.** Representative micrographs of comets derived from PBLs of healthy individuals (a), EC patients (b) and endometrial carcinoma cells (c).

To determine the possibility of using lymphocytes as surrogate cells that reflect certain characteristics of malignant cells, a comparison analysis was performed between the levels of DNA damage in PBLs and endometrial tumor cells. In this study, no association between these parameters was observed (r = 0.11; p > 0.05).

Comparison of the level of DNA damage between clinical and morphological features of EC patients did not reveal the relationship between DNA damage in PBLs and tumor cells or the degree of tumor differentiation as well as the depth of myometrial invasion (Table 1). However, it was found that the level of DNA damage in both PBLs and tumor cells depends on the body mass index (BMI) of EC patients. Thus, individuals with BMI above the median (34.1 kg/m$^2$) had significantly higher levels of DNA damage than those with lower obesity (< 34.1 kg/m$^2$) (p < 0.05) (Table 1). Therefore, increased DNA damage in PBLs was observed in patients with obesity class I (BMI 31.0–35.9 kg/m$^2$) (Table 1).

**Table 1.** Comparison of DNA damage in PBLs and tumor cells with clinical and morphological characteristics of EC patients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Level of DNA damage, % tail DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endometrial carcinoma</td>
<td>8.8 ± 3.6</td>
</tr>
<tr>
<td>Moderately differentiated</td>
<td>6.7 ± 0.5</td>
</tr>
<tr>
<td>Poorly differentiated</td>
<td>7.6 ± 1.2</td>
</tr>
<tr>
<td>BMI &gt; 30 kg/m$^2$</td>
<td>24.7 ± 1.3</td>
</tr>
<tr>
<td>BMI &gt; 25 kg/m$^2$</td>
<td>20.8 ± 2.0</td>
</tr>
<tr>
<td>BMI &gt; 20 kg/m$^2$</td>
<td>17.7 ± 1.5</td>
</tr>
<tr>
<td>BMI &gt; 15 kg/m$^2$</td>
<td>14.6 ± 1.0</td>
</tr>
</tbody>
</table>

**Fig. 2.** DNA damage in PBLs and tumor cells of EC patients with regard to family history of cancer

In order to assess the association between DNA damage level in PBLs and EC risk, the OR value among matched case and control groups (90 EC patients and 30 healthy individuals) was calculated. According to the obtained data, the median (Me) of % tail DNA in PBLs was 3.7, on the basis of which all the examined women were divided into groups with (high > Me) and low (< Me) levels of DNA damage. It was found that for women who had high level of DNA damage in PBLs, the risk of EC was greater compared to those with low level of such lesions (OR value of 3.5) (Table 3).

**Table 2.** Association between DNA damage in PBLs and EC risk

<table>
<thead>
<tr>
<th>Degree of relationship</th>
<th>Level of DNA damage</th>
<th>Group of examined women</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female offspring (n)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endometrial carcinoma</td>
<td>8.8 ± 3.6</td>
<td>8.8 ± 3.6</td>
<td>1.0 (0.5–1.8)</td>
</tr>
<tr>
<td>Moderately differentiated</td>
<td>6.7 ± 0.5</td>
<td>6.7 ± 0.5</td>
<td>1.0 (0.5–1.8)</td>
</tr>
<tr>
<td>Poorly differentiated</td>
<td>7.6 ± 1.2</td>
<td>7.6 ± 1.2</td>
<td>1.0 (0.5–1.8)</td>
</tr>
<tr>
<td>BMI &gt; 30 kg/m$^2$</td>
<td>24.7 ± 1.3</td>
<td>24.7 ± 1.3</td>
<td>1.0 (0.5–1.8)</td>
</tr>
<tr>
<td>BMI &gt; 25 kg/m$^2$</td>
<td>20.8 ± 2.0</td>
<td>20.8 ± 2.0</td>
<td>1.0 (0.5–1.8)</td>
</tr>
<tr>
<td>BMI &gt; 20 kg/m$^2$</td>
<td>17.7 ± 1.5</td>
<td>17.7 ± 1.5</td>
<td>1.0 (0.5–1.8)</td>
</tr>
<tr>
<td>BMI &gt; 15 kg/m$^2$</td>
<td>14.6 ± 1.0</td>
<td>14.6 ± 1.0</td>
<td>1.0 (0.5–1.8)</td>
</tr>
</tbody>
</table>

**DISCUSSION**

The issue of the occurrence and causes of genome instability in PBLs of cancer patients has been actively investigated over recent years. Results of several studies indicate a significant destabilization of the genome in PBLs of cancer patients. In particular, high level of baseline DNA damage was detected in patients with malignant tumors of breast, ovary, prostate, lungs, esophagus, bladder and kidney [13–20]. A number of explanations for this phenomenon have been supported in the literature. It is believed that an increased level of DNA damage in PBLs and malignant cells may be caused by influence of environmental genotoxic factors as well as substances released during metabolic processes in cancer patients [2]. Very often, the effect of such factors emerges through the action of ROS that, combined with antioxidant system failure, leads to the development of oxidative stress. ROS are characterized by pronounced DNA-damaging properties. They can induce the appearance of single- and double-stranded DNA breaks, highly mutagenic 8-oxo, 7,8-dihydro -2'-deoxyguanosine and other nucleotide modifications. It should be noted that today the genotoxic influence of ROS is considered as one of the possible mechanisms of carcinogenesis. In fact, oxidative DNA damage caused by ROS is constantly detected in malignant neoplasms of various localizations [23].

It is supposed that the main sources of endogenous ROS are the reactions of cellular respiration, lipid peroxidation and inflammation. Along with the latter, the metabolism of xenobiotics and estrogen receptor (ER) may cause a strong influence on the development of genome instability in PBLs and tumor cells in EC patients, since these processes are accompanied by the formation of reactive oxygen species. The damage caused by ROS may also be associated with the development of inflammatory cytokines and semiquinones which can directly bind DNA, leading to the occurrence of apurinic sites, or enter the redox reactions with ROS production [26–28]. It is possible that the high level of DNA damage in PBLs of EC patients may be elevated by ER-dependent effects as far as their function is an important source of estrogen. In addition, elevated BMI values are related to increased oxidative stress and estrogen receptor (ER)-dependent cytokines such as substances released during metabolic processes in cancer patients [2].

An increased level of DNA damage in tumor cells in EC patients with family history of cancer can be caused by hereditary defects in DNA repair genes and tumor suppressor genes. In terms of this issue, it is assumed that there are other molecular mechanisms that determine hereditary predisposition to EC, which may also be associated with the development of genome instability [32, 33]. Thus, it was found that the risk of developing hormone-dependent tumors...
depends on the polymorphism of the genes involved in the estrogen metabolism [34, 35]. According to Santos et al., some polymorphic variants of such genes can modulate the level of chromosomal instability in PBLs of cancer patients [35].

It should be noted that the issue of the underlying causes of genome instability in cells of cancer patients remains open today. There is an opinion that genome instability can precede tumorigenesis as well as it may be the consequence of this process. Actually, the development of a malignant disease may enhance the level of pre-existing genome destabilization in non-malignant cells through the effect of genotoxic substances released by the tumor on adjacent tissues and distant organs [23–26].

In conclusion, the results of the study indicate a pronounced destabilization of the genome in PBLs and tumor cells of EC patients, which may reflect homeostasis disorders in these women. It was shown that in both PBLs and tumor cells, the level of DNA damage depends on the BMI of EC patients and increases in cases with elevated values of this parameter. In addition, the dependence of the level of DNA damage in endometrial carcinoma cells on familial aggregation of cancers was detected: the amount of DNA lesions was higher in EC patients with family history of cancer. The presented data indicate the possibility of determining the risk of EC by DNA damage in PBLs of cancer patients [35].


REFERENCES


Copyright © Experimental Oncology, 2017