In vitro sensitivities to UVA of lymphocytes from patients with colon and melanoma cancers and precancerous states in the micronucleus and the Comet assays

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To use lymphocytes as surrogate cells to investigate their in vitro sensitivities to ultraviolet (UV) treatment in different cancers and precancerous states by comparison with lymphocytes from healthy control individuals was the main aim of this research. UV light induces precise cellular and genomic mutations. In this study, the effect of ultraviolet A (UVA) (320–400 nm) was used as a generic mutagen to evaluate in vitro different sensitivities from lymphocytes of patients with suspected melanoma (SM), malignant melanoma (MM), polyposis coli (PC) and colorectal cancer (CRC). DNA damage was evaluated by two different methods: the micronucleus (MN) assay and the Comet assay. The baseline frequency of MNs was significantly increased in lymphocytes from all patients (SM, MM, PC and CRC) when compared to healthy individuals. After UV irradiation, MN frequencies were significantly increased in lymphocytes of all groups, both patients and healthy individuals. However, the MN frequency in all patient groups was significantly higher than in the healthy individual group. Similar results for the induction of genomic DNA damage were obtained for the Comet assay. Also for the Comet assay, UVA-induced DNA damage for all four patient groups was significantly increased when compared to healthy individuals (SM, MM, PC and CRC groups: P < 0.001). Conclusively, peripheral lymphocytes from patients with cancers MM and CRC or precancerous states SM and PC are more sensitive to a generic mutagen such as UVA than lymphocytes from healthy individuals. This feature may be used as an essential biomarker to screen and diagnose precancerous states and cancers in early stages.

Introduction

Oxidative DNA damage to cells is an unavoidable consequence of cellular metabolism; however, interactions with exogenous sources such as carcinogenic compounds, redox-cycling drugs, ionising and ultraviolet (UV) radiation also contribute to oxidative damage (1,2).

One of the most commonly diagnosed cancers in humans is colorectal cancer (CRC) (3). Around 106 new cases of CRC are diagnosed each day in the UK and it is the third most common cancer after breast and lung cancers. In 2007, there were 38 608 new cases of large bowel cancer registered in the UK with around two-thirds (24 274) in the colon and one-third (14 334) in the rectum (4,5). Two major sources of DNA damage contributing to progression to cancer appear to be DNA-adduct-forming molecules and reactive oxygen/nitrogen species (ROS/RNS) (1). Certain risk factors to increase the chance of developing CRC are inflammatory bowel disease (IBD), colonic adenomas, a family history of CRC and a high-fat/low-fibre diet. However, the precise aetiology of CRC is unknown and environmental, genetic and dietary factors are believed to be responsible for 85–90% of all cases (6,7). In CRC pre-states, most frequently metaplastic polyps can be found, which are very similar in appearance to adenomatous polyps but have the potential to become malignant. Houston et al. (8) were able to identify four new loci predisposing individuals to CRC and the (loci) are at 14q22.2, 16q22.1, 19q13.1 and 20p12.3. Inflammation is the body’s natural reaction to invasion by an infectious agent to a physical or chemical insult or to traumatic damage, and chronic inflammation and infection can be major causes of cancer (9).

Dysplastic naevi (DN) are a known risk factor for malignant melanoma (MM) and another common type of cancer categorised as a pre-cancerous suspected melanoma. Within DN cells, higher concentrations of ROS and DNA damage can be found than in normal skin melanocytes from the same individuals (10). Cutaneous MM is the most lethal form of skin cancer, and over 8000 cases have recently been diagnosed each year in the UK and the numbers are steadily rising faster than any other cancer (11). UV-induced damage is involved in the initiation of MM as melanocytes seem to be extremely susceptible to free radicals (12,13). UVB (290–320 nm) exposure was thought to be responsible for non-melanoma skin cancer as it generates cyclobutane pyrimidine dimers (CPDs) in skin cells, which in turn can develop into cutaneous squamous cell or basal cell carcinomas (14). By the increasing usage of ultraviolet A (UVA) tanning beds and the parallel rise in the development of melanomas, the adverse effects of UVA exposure (320–400 nm) was also suggested (14). Like UVB, UVA can cause non-melanoma skin cancers, but tumours take longer to develop and require much higher doses. UVA-induced skin cancers have been thought to derive from indirect damage to DNA caused primarily by the generation of reactive oxygen intermediates (14).

It has been recently found that cancer itself and most likely its precancerous states can cause stress to the entire organism (15). This fact may then lead to differences in sensitivity to exogenous genotoxic insults in cells unrelated to the cancer,
such as peripheral lymphocytes. Insults due to environmental stressors or lifestyle factors may subsequently lead to higher damage in sensitive cells (16).

In this study, we investigated in vitro the differential responses of cells to UVA, a physical generic mutagen, by exposing human peripheral lymphocytes originating from patients with two common but different human cancers, CRC and MM and their precursor stages, i.e. polyposis coli (PC) and SM. Two different assays, the cytokinesis-block micronucleus (CBMN) assay and the Comet assay were used to assess induction of MNs and DNA genomic integrity.

Materials and methods

**Blood samples**

Peripheral whole blood samples were collected by venepuncture from over 200 male and female individuals (100 patients and 100 healthy controls) each for the MN and Comet assays (four groups: 40 for MM and 20 individuals for the rest of the groups SM, CRC and PC, each group with corresponding negative controls). According to molecular epidemiology which is a contribution of potential genetic and environmental risk factors, identified at the molecular level, to the aetiology, distribution and prevention of disease within families and across populations. The number of samples, as advised by the statistician in the present study, has previously had sufficient statistical power to obtain highly statistically significant results (17). Ages of individuals ranged from 20 to 78 years old for the patients’ groups and from 24 to 58 years old for the healthy control group. MM and SM patients were receiving surgery as a treatment at the Dermatology Department of the St Luke’s Hospital in Bradford, UK. CRC and PC patients were untreated or only treated with surgery at the Colorectal Surgery Department of St. Luke’s Hospital and Bradford Royal Infirmary (BRI), Bradford, West Yorkshire, UK. One hundred healthy volunteers were recruited within the Division of Medical Sciences at the University of Bradford (West Yorkshire, UK). The smoking status of the study population is given in Table I. Fuller description of the population characteristics is provided as Supplementary Information (available at Mutagenesis Online). Ethical permission was obtained from both the BRI Local Ethics Committee (reference numbers 04/QI202/132 and 04/Q1202/15) and the University of Bradford’s subcommittee of Research Ethics involving Human Subjects (reference number 0405/8). Selecting the patients was based on the onset of their disease and the type of their cancer.

**Questionnaire for patients and controls**

A questionnaire was administered to each donor immediately after taking the blood sample. The completed questionnaire for the patient and control groups provided essential information about lifestyle, confounding factors and medical treatment or any other drug intake. Information regarding internal endogenous factors (gender and age) and external exogenous factors (intake of medicines and tobacco, smoking habits and diet) were collected via an ethically approved clinical questionnaire. The type of cancers and polyps and also the location of these two types of polyps were included in the study. The location of cancer lesions was not specified. However, it was necessary to understand the pathological type of cancer, e.g. adenocarcinoma or squamous carcinoma as this is indicative of the position.

**UVA source**

For UVA exposure, a table top lamp housing two 15W PUVa tubes (Waldmnn, Villingen-Schwenningen, Germany; bought from Athrodax Healthcare International Ltd, UK) was used. The spectrum of the PUVa tube ranged from 320 to 410 nm with a maximum at 351 nm. The lamp was positioned 15 cm above the cell layer. For the MN assay, the cells were irradiated in suspension in Corning 25 cm² plastic culture flasks (WVR, UK), placed horizontally on the bench under the UV lamp. For the Comet assay, cells were exposed embedded in 0.5% agarose on slides.

**The CBMN assay**

After collection, whole blood (5 ml) from each of the patients and healthy volunteers were cultured on the same day as collection in 25 cm² culture flasks with Rotherwell Park Memorial Institute standard prepared medium (18). After 24 h, the cells were ready for the treatment. There were three different groups of treatment. Group one received no treatment as a negative control group, group two was treated with mitomycin C (MMC) (Sigma, UK) 0.4 lµl per flask as a positive control and group three to six from each group of patients (MM, SM, CRC and PC) and healthy control exposed to UVA for 30 min at a mean surface intensity of 1.53 ± 0.01 mWcm². Two slides per culture were prepared for each experiment and cultured twice on separate occasions, dried overnight and stained with filtered 5% Giemsa (Merck, Darmstadt, Germany) in pH 6.5 Sorensen buffer. All chemical reagents not specified otherwise were obtained from VWR. The slides were evaluated for quality, then mounted with coverslips and coded. The slides were scored according to the criteria defined by Fenech et al. (19). MNs were evaluated in 1000 binucleated lymphocytes (BiMN) per treatment. Duplicate cultures were used, two slides per culture were prepared for each experiment and cultured twice on separate occasions, so 2000 binucleated were in fact scored.

Additionally, nuclear bridges (BiNPs) and nuclear buds (BiBuds) were assessed in binucleated cells as well as MNs in mononucleated cells (MonoMN). The cytokinesis-block proliferation index (CBPI) was calculated from a total of duplicated 1000 mononucleonated, binucleated and multinucleated cells per treatment. The CBPI is defined as CBPI = [(number mononucleated cells) + (2 × number binucleated cells) + (3 × number multinucleated cells)]/total number of cells (20).

**The alkaline Comet assay**

Lymphocytes were isolated from whole blood of all patients and healthy individuals using Lymphoprep according to the manufacturer’s protocol (Axis Shield, Norway). Lymphocytes were treated with UVA for 15 min at 37°C at a mean sample surface intensity of 1.53 ± 0.01 mW cm². Untreated lymphocytes served as a negative control. Twenty patients of each SM, MM and 20 patients of each PC, CRC and 20 HCIs were used for the investigation.

After the treatment, the cells were centrifuged for 5 min at 9000 g. For DNA damage studies with the Comet assay, the cell suspension was mixed with the same volume of 1% low melting point agarose. The slides were evaluated for quality, then mounted with coverslips and coded. The slides were scored according to the criteria defined by Fenech et al. (19). MNs were evaluated in 1000 binucleated lymphocytes (BiMN) per treatment. Duplicate cultures were used, two slides per culture were prepared for each experiment and cultured twice on separate occasions, so 2000 binucleated were in fact scored.

Additionally, nuclear bridges (BiNPs) and nuclear buds (BiBuds) were assessed in binucleated cells as well as MNs in mononucleated cells (MonoMN). The cytokinesis-block proliferation index (CBPI) was calculated from a total of duplicated 1000 mononucleonated, binucleated and multinucleated cells per treatment. The CBPI is defined as CBPI = [(number mononucleated cells) + (2 × number binucleated cells) + (3 × number multinucleated cells)]/total number of cells (20).

**Statistics**

For the CBMN assay, the numbers of MonoMN and BiMN, nuclear buds, nucleoplastic bridges, CBPI and percentage of binucleated cells were used as dependent variables. The different doses were considered as independent variables. The post hoc analysis Fisher’s Least Significant Difference test was used to determine the significant differences between group means. The level of

<table>
<thead>
<tr>
<th>Group</th>
<th>Numbers</th>
<th>Number of cigarettes/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy controls</td>
<td>17</td>
<td>Under 10</td>
</tr>
<tr>
<td>MM</td>
<td>7</td>
<td>Under 10</td>
</tr>
<tr>
<td>SM</td>
<td>6</td>
<td>Under 10</td>
</tr>
<tr>
<td>CRC</td>
<td>0</td>
<td>Under 10</td>
</tr>
<tr>
<td>PC</td>
<td>3</td>
<td>Under 10</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>10–20</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>More than 20</td>
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<tr>
<td></td>
<td>1</td>
<td>More than 20</td>
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<tr>
<td></td>
<td>2</td>
<td>More than 20</td>
</tr>
</tbody>
</table>

Cell viability check

Cell viability at the concentrations chosen for each experiment was checked after treatment. Viability was determined by Trypan blue exclusion (10 µl of 0.05% Trypan blue was added to 10 µl of cell suspension) and the percentage of cells excluding the dye was evaluated using a haemocytometer. Viabilities between 75 and 95% were accepted for use in studies to avoid results from cytotoxicity (22).

Statistics

For the CBMN assay, the numbers of MonoMN and BiMN, nuclear buds, nucleoplastic bridges, CBPI and percentage of binucleated cells were used as dependent variables. The different doses were considered as independent variables. The post hoc analysis Fisher’s Least Significant Difference test was used to determine the significant differences between group means. The level of
statistical significance was set at 5% ($P < 0.05$). The statistical package STATISTICA 6.0 (IL, USA) was employed. For the Comet assay, Gaussian normality was violated for many of the scale variables as endorsed by the Kolmogorov–Smirnov test. Therefore, non-parametric test procedures were adopted wherever necessary, such as the Kruskal–Wallis (K–W) and the Mann–Whitney (M–W) tests for independent samples. When testing intra-subject differences in DNA damage, the Wilcoxon Signed Rank test was applied.

Results

Population characteristics

These are provided as Supplementary Information (available at Mutagenesis Online).

Confounding factors

Ethnicity, age, gender, smoking and drinking habits. There were small differences of median levels of DNA damage in Caucasians ($n = 81$) and Asians ($n = 19$) as well as in males and females in the healthy control group. However, these differences were not found to be statistically significant (M–W, $P = 0.140$). There were also no statistically significant differences in the age distributions between patients as well as between control individuals (patients group’ mean age = 48.3 years ± SD 10.2, control individuals’ mean age = 36.8 years ± SD 7.4). No major differences were seen due to smoking (Table I) and/or drinking habits. In the control group, the distribution of gender was 34% for females. Comparing to MM and SM, the percentage was almost 50%. Although in the PC and CRC groups, the distribution went back to 35 and 30% similar to the control group, respectively.

Previous medication in the patient groups as a confounding factor. As mentioned earlier at the time of collecting blood samples, no cancer patients had received any chemotherapy but some had had surgery.

MN assay

Healthy individual group. After UVA treatment, lymphocytes of healthy individuals showed a statistically significant reduction in CBPI from 1.84 to 1.62 for UVA ($P < 0.001$) and to 1.67 for MMC ($P < 0.01$) due to a significant decrease in binucleated cells (BiNC) as seen in Table II. Additionally, UVA treatment significantly induced MNs in cytokinesis-blocked lymphocytes almost to the levels of the positive control MMC. The number of binucleated cells containing MN increased 2.8-fold (BiMN; $P < 0.001$) (Figure 1) but also the number of mononucleated cells with MN increased significantly by 4-fold (MonoMN; $P < 0.01$). However, there were no statistically significant differences in the number of nucleoplasmic bridges and nuclear buds (BiNPB and BiBud) found in BiNC (Table II).

SM and MM patient groups. For both groups, the proliferation index CBPI was significantly decreased from 1.61 (SM group) and 1.73 (MM group) to 1.47 and 1.52, respectively, after UVA treatment (both $P < 0.01$) and to 1.36 ($P < 0.001$) and 1.67 ($P < 0.05$), respectively, after MMC treatment (Table II). Similarly, this was also observed for the percentage of BiNC in

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**Table II.** The effect of UVA treatment on peripheral blood lymphocytes from SM, MM, PC and CRC patients compared to HCs

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CBPI</th>
<th>%BiNC</th>
<th>BiMN</th>
<th>BiNPBs</th>
<th>BiBuds</th>
<th>MonoMN</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCs</td>
<td>1.84 ± 0.08</td>
<td>56.03 ± 5.46</td>
<td>9.86 ± 2.6</td>
<td>0.00 ± 0.00</td>
<td>0.93 ± 0.97</td>
<td>2.71 ± 0.97</td>
</tr>
<tr>
<td>UVA</td>
<td>1.62 ± 0.04***</td>
<td>41.80 ± 3.84***</td>
<td>27.50 ± 1.02***</td>
<td>0.60 ± 1.05</td>
<td>0.86 ± 0.67</td>
<td>10.79 ± 5.47***</td>
</tr>
<tr>
<td>MMC</td>
<td>1.67 ± 0.02**</td>
<td>47.60 ± 3.26**</td>
<td>32.71 ± 3.54**</td>
<td>0.14 ± 0.87</td>
<td>1.43 ± 0.98**</td>
<td>12.00 ± 6.89**</td>
</tr>
<tr>
<td>SM patients</td>
<td>1.61 ± 0.02§§</td>
<td>49.70 ± 2.03§§</td>
<td>23.00 ± 1.57</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>2.00 ± 1.76</td>
</tr>
<tr>
<td>UVA</td>
<td>1.47 ± 0.03</td>
<td></td>
<td></td>
<td>37.90 ± 1.42</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMC</td>
<td>1.36 ± 0.02</td>
<td></td>
<td></td>
<td>30.30 ± 3.45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MM patients</td>
<td>1.73 ± 0.04§</td>
<td>52.30 ± 3.75§</td>
<td>22.00 ± 1.89§§</td>
<td>0.20 ± 1.42</td>
<td>4.20 ± 0.87</td>
<td>4.20 ± 3.64§§</td>
</tr>
<tr>
<td>UVA</td>
<td>1.52 ± 0.03</td>
<td></td>
<td></td>
<td>46.50 ± 1.78</td>
<td></td>
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<tr>
<td>MMC</td>
<td>1.67 ± 0.03</td>
<td></td>
<td>49.70 ± 2.34</td>
<td></td>
<td>45.89 ± 2.85</td>
<td></td>
</tr>
<tr>
<td>PC patients</td>
<td>1.63 ± 0.06§§</td>
<td>43.43 ± 4.46§§</td>
<td>13.41 ± 4.25§</td>
<td>0.30 ± 0.75</td>
<td>1.60 ± 1.33§</td>
<td>4.80 ± 2.67§</td>
</tr>
<tr>
<td>UVA</td>
<td>1.38 ± 0.03</td>
<td></td>
<td></td>
<td>31.13 ± 3.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMC</td>
<td>1.45 ± 0.03</td>
<td></td>
<td></td>
<td>37.35 ± 2.87</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRC patients</td>
<td>1.61 ± 0.04§§</td>
<td>42.88 ± 2.56§§</td>
<td>23.89 ± 1.66§§</td>
<td>0.20 ± 0.00</td>
<td>2.50 ± 1.79§§</td>
<td>3.60 ± 2.63§</td>
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<tr>
<td>UVA</td>
<td>1.31 ± 0.03</td>
<td></td>
<td></td>
<td>26.04 ± 1.65</td>
<td></td>
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</tr>
<tr>
<td>MMC</td>
<td>1.51 ± 0.02</td>
<td></td>
<td>30.30 ± 1.73</td>
<td></td>
<td>51.00 ± 1.45</td>
<td></td>
</tr>
</tbody>
</table>

* , †, §, $P < 0.05$, ** , ‡, §§, $Y P < 0.01$, *** , §§§, $Y Y P < 0.001$; * indicates the level of statistical significance when comparing lymphocytes from healthy individuals after treatment with UVA and MMC to untreated lymphocytes; † indicates the level of statistical significance when comparing lymphocytes from patients after treatment with UVA and MMC to untreated lymphocytes; § shows the level of statistical significance when comparing untreated lymphocytes from patients to healthy controls; $Y$ shows the level of statistical significance when comparing treated lymphocytes from patients to treated healthy controls.

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UV, malignant melanoma, colorectal cancer
1000 scored cells (mono-, bi- and multinucleated). UVA treatment also induced cytogenetic damage significantly increasing the frequency of BiNC carrying MN. The significant increase of 1.1-fold ($P < 0.05$) for the SM group and 1.9-fold ($P < 0.01$) for the MM group did not reach the increase in damage as shown by the positive control compound MMC of more than 2-fold ($P < 0.05$ and $P < 0.001$, respectively) (Table II). For SM patients, the frequency of MonoMN significantly increased by 3.5-fold ($P < 0.05$). This was not seen for lymphocytes from MM patients after UVA treatment. MMC treatment resulted only in a significant increase in MonoMN in the MM group (2.5-fold, $P < 0.05$). Although there was no statistically significant increase in the observed BiNPB, the nuclear buds found in BiNC increased significantly. For the SM group, while the UVA treatment did not induce any BiBud, MMC treatment did (4 in 1000 BiNC, $P < 0.01$). For the MM group, UVA as well as MMC treatment resulted in significant increases in frequencies of BiBud from 4 per 1000 BiNC to around 6.5 per 1000 BiNC ($P < 0.05$).

When both groups were compared to the group of healthy individuals (Table II and Figure 1), untreated lymphocytes from SM and the MM patients showed significantly lower CBPI frequencies, from 1.84 to 1.61 ($P < 0.01$; SM group) and to 1.73 ($P < 0.05$; MM group), which can also be seen in the reduction of the percentage of BiNC. Additionally, the baseline cytogenetic damage (induced BiMN) was significantly reduced from 1.84 (SM group) to 1.61 (CRC group) to 1.38 and 1.31, respectively (Table II and Figure 1). Frequencies of BiNPB did not show any differences to those of the healthy individual group. However, BiBud and MonoMN were significantly increased in both groups by 1.7-fold ($P < 0.05$) and 2.7-fold ($P < 0.01$), respectively, for the PC group and by 1.8-fold ($P < 0.05$) and 1.3-fold ($P < 0.01$), respectively, for the CRC group.

In this study, ethnicity, age, gender, smoking and drinking habits as confounding factors were considered, although there were no statistical significant differences by comparison with the healthy individual control group.

**Comet assay**

**SM and MM patient groups.** UVA treatment of lymphocytes from patients with SM and MM showed significantly increased DNA damage 1.9-fold and 2.3-fold (both $P < 0.001$), respectively, when compared to untreated lymphocytes (Figure 2). In addition, before treatment with UVA, the lymphocytes from SM and MM patients showed significantly more damage (a 6-fold increase) when compared with those from the healthy controls (both $P < 0.01$) (Figure 2). However, no significant differences in DNA damage were detected in lymphocytes from SM and MM after treatment with UVA except when they were the healthy control group.

**PC and CRC patient groups.** UVA treatment significantly induced DNA damage ($P < 0.001$) in lymphocytes from all three groups (healthy individuals, PC and CRC). The measured OTM increases were 3.2-fold for the PC group, 3-fold for the CRC group and 3.3-fold for the healthy individual group. The general DNA damage in lymphocytes from CRC patients before UVA treatment was highest followed by PC patients and then HClis, after treatment with UVA (Figure 3). After inducing the DNA damage to lymphocytes from CP and CRC patients compared to the HCl group the OTM of the patient groups increased significantly 1.38-fold for PC and 1.48-fold for CRC ($P < 0.05$) (Figure 3). Additionally, the lymphocytes of the patient groups (PC and CRC) before treatment with UVA by comparing to the HCl showed increased 1.43-fold for PC and 1.6-fold for CRC ($P < 0.05$) (Figure 3).

Alternatively, the tail length was checked besides the OTM. There was a significant increase in both groups (controls and patients) after treating with UVA ($P < 0.001$).
level of statistical significance when comparing untreated lymphocytes from patients after treatment with UVA and MMC to untreated lymphocytes; § shows the level of statistical significance when comparing untreated lymphocytes from patients to healthy controls.

Discussion

Different sensitivities of body cells to genotoxins can be determined in peripheral blood lymphocytes. These cells are ideal to be used as surrogates for all other body cells as DNA is basically the same in all cells and assays to detect genotoxic DNA damage are used in this study (Figure 1). It is of little relevance that the cells do not divide as they are stimulated to divide in the MN assay. They are the most accessible cells and the blood flow integrates the systemic response for cancer detection. Also these cells as surrogates have been used in the field of Human Monitoring for the last 40 years (18). As peripheral lymphocytes are exposed to various environments inside the body by travelling in the blood stream, they reflect a common denominator of endogenously and exogenously induced damage from chemical and physical genotoxic insults such as food mutagens or oxidative stress. We have previously shown that treatment with hydrogen peroxide and the food mutagen IQ (2-amino-3-methylimidazo[4,5-f]-quinoline) induced more DNA damage in lymphocytes from patients with IBD, a disease prone to high levels of ROS, than in those from healthy individuals (16). Irrespective of their underlying condition, patients with IBD on the other hand have an increased risk of developing CRC (23).

In this study, we assessed in vitro the different sensitivities of peripheral lymphocytes from patients diagnosed with two very common types of cancers, MM and CRC or with their precancerous states, SM and PC. The patients in all groups did not have any course of chemotherapy or radiotherapy; thus, the effect of their background treatment was not encountered in our study. Our results showed that lymphocytes from selected precancerous states (SM and PC) and cancers (MM and CRC) had significantly decreased CBPI values, hence, a lower mitotic index as well as a significantly increased frequency of induced MNs in binucleated cells, i.e., genetic damage, when compared to HClis (Table II and Figure 1). This effect was confirmed by examining the lymphocytes from SM, MM and PC, CRC and treating them with UVA and compared to healthy controls individuals in vitro in the Comet assay (Figures 2 and 3). These findings have been recently supported as newly diagnosed cancer patients were found to have elevated levels of DNA damage in peripheral blood lymphocytes (15). Levels of reactive oxidative and reactive nitrogen species in relation to the antioxidant status are most likely to be one of the reasons for this observed damage as they can transiently or permanently damage nucleic acids, lipids and proteins and therefore can increase cancer risk (24,25).

To induce DNA damage in vitro in lymphocytes from our selected patient groups, UVA was used as a generic mutagen. Compared to a chemical genotoxin, the advantage of using UVA light was the exact setting of exposure time and strength of the genotoxic insult. UVA is part of the sunlight with its electromagnetic spectrum at sea level (290–5000 nm) not only including the visible (56%) and infrared (39%) part but also UV light (5%) (26). Mostly UVA (320–400 nm) and to a lesser extent due to atmospheric absorption UVB (290–320 nm) reaches the earth’s surface, while the germicide UVC part is completely filtered off. The UVA/B light has been commonly characterised as an environmental human carcinogen being also responsible for erythema (sunburn), tanning, photo-aging and immune suppression. However, artificial UV sources, mainly emitting UVA, can also be found in tanning studios and for the treatment of psoriasis (27,28). Absorption of UVA in tissue results in the generation of reactive oxygen and nitrogen species and labile iron, which can in turn damage other biomolecules such as DNA (29,30). The most frequent type of DNA damage after UV exposure are CPD being mostly contributed by marginal amounts of UVB, while UVA-induced oxidative DNA damage like 8-oxo-guanine depends on non-DNA chromophores present in the cells (31).

For the DNA damage examined after in vitro UVA treatment, our results showed that peripheral lymphocytes from patients diagnosed with the precancerous states and the associated cancer have a significantly higher sensitivity to the genotoxic insult when compared to healthy individuals (Table II and Figures 1–3). The mitotic index CBPI significantly dropped by up to 19% compared to 12% for the group of healthy individuals. Interestingly, the decline in CBPI was smaller for the groups of precancerous states and the highest for the CRC group. For the PC/CRC groups, the decline in CBPI was even lower than that for the positive control (MMC treatment). After inducing DNA damage by UVA in peripheral lymphocytes from SM, MM patient groups and healthy individuals, the differences between patient groups and the HCl were detected ($P < 0.001$). In addition, for the PC and CRC patient groups compared to the HCl group an increase $P < 0.001$). In addition, for the PC and CRC patient groups compared to the HCl group an increase after treatment with UVA was shown in the Comet assay ($P < 0.05$) (Figures 2 and 3).

MM is a life threatening type of skin cancer. It originates from UV-induced DNA damage in specialised skin cells, the melanocytes. This initial damage causes mutations, which can lead to melanoma. However, the mechanism underlying the role of UV light exposure from sunlight in the aetiology of cutaneous MM is unclear. In xeroderma pigmentosum, a disease with severe sensitivity to UV, due to a defect in nucleotide excision repair, there is a high incidence of MM, suggesting that DNA repair capacity (DCR) plays a role in sunlight-induced MM (32). Also, DCR reduction is one of the risk factors for MM and may have a separate role in susceptibility to sunlight-induced MM among healthy people (32). The interplay between genetic factors and the UV spectrum of sunlight such as specific signal transduction pathways that regulate cell cycling.
differentiation and apoptosis is one of the mechanisms of UV-induced skin cancers. Another mechanism includes mutations in genes coding for proteins in the Hedgehog pathway and in the p53 gene (33).

For the induced genetic damage in our study, the induction of BiMN after UVA treatment was higher in lymphocytes from cancer and precancerous state patients when compared to the control group. Only those from patients of the precancerous SM group were lower but still significantly increased. When compared to the genotoxic impact of MMC, UVA treatment produced generally lower frequencies of induced MNs (Table II). Especially for the PC/CRC group of patients, the significantly increased induction of MNs in mononucleated cells was obvious (Table II) \((P < 0.001)\) in the CRC group after UVA induction. Diseases like cancer have been shown to cause cellular instability increasing chromosomal damage seen in an increase in induced MNs using the MN assay (34). The genomic damage to the lymphocytes of peripheral blood has been widely used as a biomarker of genotoxic environmental factors, and long-term studies have demonstrated its validity and high clinical productivity (35).

In conclusion in our study, peripheral blood lymphocytes were used as surrogate cells to characterise induced DNA damage after a genotoxic insult. Induced MNs, the change in the mitotic index and the change in DNA integrity, assessed by the Comet parameters, served as biomarkers for genotoxic damage. This study shows that peripheral lymphocytes from patients diagnosed with precancerous states and with the associated cancer treated in vitro with UVA are expressing different sensitivities to a genotoxic insult when compared to a HGI group. Thus, the damage afflicted by UVA was significantly higher in lymphocytes, which originated from individuals with cancer or a precancerous state. Our findings suggest that cells not in close proximity to the cancer or the precancerous lesions might carry higher intrinsic damage due to for instance increased oxidative stress and are therefore more sensitive to genotoxic insults. This feature of differential sensitivity of lymphocytes from healthy individuals and patients with cancers or precancerous states could be used as a biomarker in the screening and diagnosis of precancerous states and cancers in the early stage.

Supplementary data
Supplementary Information is available at Mutagenesis Online.

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References