Ann. Ist. Super. Sanità
Vol. 25, N. 1 (1989), pp.81-90

MONITORING OF GENETIC INSTABILITY IN SUBJECTS WITH INCREASED RISK OF CANCER

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Summary. - *Results of investigations performed to obtain data on genomic instability in subjects with increased risk of cancer are reported. People at risk for environmental or genetic factors included in this study were: 1) individuals from the Seveso population exposed to dioxin; 2) psoriatic patients exposed to photochemotherapy; 3) members of a family with a high incidence of colonic cancer; 4) patients affected by nevoid basal cell carcinoma syndrome; 5) patients with photosensitivity. The following cellular parameters were analyzed in peripheral blood lymphocytes: i) frequency of chromosome aberrations and of sister chromatid exchanges; ii) sensitivity to different DNA damaging agents; iii) efficiency of DNA repair after UV irradiation and iv) frequency of spontaneous mutations.*

Riassunto (Monitoraggio della instabilità genetica in individui a rischio per lo sviluppo di tumori). - *Sono riportati i risultati di ricerche eseguite allo scopo di ottenere dati su parametri cellulari indicatori di instabilità genetica in individui a rischio per lo sviluppo di tumori. I tests applicati utilizzando linfociti di sangue periferico valutano la frequenza di aberrazioni cromosomiche e di scambi tra cromatidi fratelli, la sensibilità a mutageni che inducono tipi diversi di danni sul DNA, l'efficienza della sintesi riparativa stimolata da irradiazione con luce UV, la frequenza di mutazioni spontanee. I campioni di individui selezionati per rischio genotossico, determinato da fattori genetici o ambientali, sono i seguenti: 1) individui appartenenti alla popolazione di Seveso esposti alla diossina; 2) pazienti affetti da psoriasi trattati con fotochemioterapia; 3) individui appartenenti ad una famiglia con alta incidenza di adenocarcinoma del colon; 4) pazienti affetti da sindrome nevobasocellulare; 5) pazienti con fotosensibilità.*

Introduction

It is well established that cancer is the result of mutational events in somatic cells [1], and that chromosome mechanisms (somatic recombination, mitotic non-disjunction) as well as chromosome aberrations (deletions, translocations) represent a critical step of the pathogenesis of most human leukemias, lymphomas and also of solid tumors [2-6].

It has been suggested that in the human population various degrees of genetic instability exist that determine different degrees of predisposition to the occurence of mutant cells and then of transformed malignant cells [7]. There are subjects that may be considered genetically stable: in fact, among people exposed accidentally, occupationally and by lifestyle to genotoxic agents, not all develop tumors. On the other hand, there are persons greatly susceptible to the genotoxic effects of DNA-damaging agents. A high degree of genetic instability has been clearly demonstrated in a number of rare inherited disorders with distinctive clinical symptoms, but sharing an increased risk of cancer [8-13]. The disorders are: xeroderma pigmentosum (XP), ataxia telangiectasia (AT), Nijmegen syndrome (NBS), Bloom syndrome (BL), Fanconi anemia (FA), Werner syndrome (WS).

In patients affected by XP or by one of the "chromosome breakage syndromes" the proneness to neoplastic transformation is accompanied by cellular hypersensitivity to mutagens, chromosome fragility, DNA processing abnormalities [14].

Hypersensitivity to UV light is present also in patients affected by Cockayne syndrome (CS), that for this reason may be considered a disease with genetic instability although there is no clear evidence for increased tumor incidence [15].

In each of the mentioned diseases the abnormal phenotype has been related to the homozygous condition for a mutation affecting a specific locus. All these genetic constitutions may predispose to mutations and/or chromosome lesions, with cancer as their outcome.

Five of these diseases are genetically heterogeneous: complementation analysis demonstrated that at least ten mutations are responsible for XP, five for AT, three for CS, two for FA and NBS [16-20].

This variety of genetic changes in different disorders, each regarded as a single clinical entity, is not unexpected owing to the multiplicity and complexity of functions from which the genomic stability depends; it is

likely indeed that the genetic control of such functions requires a number of genes greater that those identified up to now.

As an alternative to single recessive mutations, an oligogenic model of inheritance has been hypothesized for diseases associated with defective DNA repair [21]. Accordingly, more than one genetic locus is involved in each patient and the abnormal phenotype is expressed only in individuals homozygous for defective alleles at more than one of a specific set of loci. As a consequence, the number of carriers of mutant co-recessive alleles involved in DNA repair processes would be many times greater than that expected for autosomal recessive inheritance. Thus, most normal individuals are likely to have a minor defect in their DNA repair mechanisms causing a hidden genetic instability and predisposition to develop cancer.

Alterations of cellular features reflecting genetic instability seem to be absent in the parents of patients affected by XP, BS, WS. In fact carriers of defective alleles for the mentioned cancer-prone syndromes are clinically asymptomatic. Conversely, some obligate carriers of AT and FA genes can be distinguished from healthy individuals by higher sensitivity to DNA-damaging agents and chromosome fragility [17, 22, 23]. Interestingly, epidemiological data on AT families suggest that the AT gene/s may contribute significantly to cancer incidence in the general populations [24].

The investigation of cellular characteristics considered indicators of genetic instability may lead to the identification of subjects at risk of genetic damage. To this purpose studies on the frequency of chromosome aberrations and sister chromatid exchanges (SCE), sensitivity to different DNA damaging agents, efficiency of DNA repair, mutability, may provide a wide spectrum of significant data.

Mononucleated cells from peripheral blood are the best cell system for this study. From the same blood sample it is possible to obtain, immediately at withdrawing or after mitogen stimulation, synchronized or partially synchronized cell populations at different phases of the cell cycle (G_0, G_1, S, mitosis) suitable for different parallel assays.

We report here a brief description of the tests used and some examples of investigations performed during the last few years in our laboratory with the aim to obtain, from the same blood sample, data on different cell parameters indicators of genotoxic damage.

Tests to detect genetic instability

Chromosome anomalies and sister chromatid exchanges (SCE)

In cytogenetic analysis on stimulated lymphocytes, both methodologic criteria and interpretation of experimental results are well defined [25]. Chromosome aberrations were scored on at least 100 metaphases for each subject on 48 and/or 72 h blood cultures. The frequency of SCE was determined on 20 second division mitoses for each subject in 72 h cultures containing 10 μM bromodeoxyuridine (BrdU). Cultures in which the newly synthesized DNA was labeled with BrdU were used for the evaluation of cell proliferative kinetics by counting cells undergoing one, two or more divisions.

Inhibition of duplicative DNA synthesis after mutagen exposure

The capacity of G_0 lymphocytes treated with mutagens to duplicate after mitogen stimulation gives a measure of cellular sensitivity to DNA-damaging agents and of repair activity [26]. The DNA duplication rate was measured in lymphocytes stimulated with phytohemagglutinin (PHA M) or pokeweed mitogen (PWM) after treatment of G_0 cells with agents inducing different types of DNA damage. Ethyl methanesulfonate (EMS), mitomycin C (MMC), 1-2-chloroethyl-3-cyclohexyl-1-nitrosourea (CCNU), 8-methoxy-psoralen (8 MOP) solutions were used at the following dose ranges: EMS 10^{-4} - 10^{-3} M; MMC 10^{-8} - 10^{-6} M; CCNU 2.5 x 10^{-5} - 10^{-4} M; 8 MOP 10^{-7} - 10^{-5} M. For PUVA treatment, the cultures were irradiated 30 min after 8 MOP addition with a UV (365 nm) dose of 1.5 kJ/m^2 using a Philips 54236 - E/70 bulb; control cultures received only UVA irradiation. UVC (254 nm) irradiation was performed with a Philips TUV 15 Watt lamp, giving a dose rate of 2 J/m^2/sec. Samples of 2 x 10^5 cells were exposed to mutagens and cultured in microtiter wells in medium supplemented with 20% calf serum as previously described [27]. 64 h after UV irradiation or mutagen exposure ^{3}H-thymidine (^{3}H-TdR, specific activity 2 Ci/mmol) was added at a final concentration of 2 μCi/ml and the incorporated radioactivity was measured 24 h later.

UV-induced unscheduled DNA synthesis (UDS)

Methods for the evaluation of sensitivity to DNA damaging agents through the analysis of unscheduled DNA synthesis (UDS) in G_0 and stimulated lymphocytes, have been validated and applied by a number of laboratories [26-31]. We analyzed the UV-induced UDS as previously described [27]. Briefly, G_0 or stimulated lymphocytes were irradiated with UV light (254 nm) and then incubated at 37 °C in the presence of 2.4 mM hydroxyurea (hu); after 15 min, ^{3}H-TdR (10 μCi/ml, specific activity 20-25 Ci/mmol) was added and the incubation continued for 1 up to 6 h. Samples without hu were treated in parallel. Incorporated radioactivity was measured as described elsewhere [27]. Repair synthesis was expressed as the difference between the incorporation values in the presence of hu in irradiated and unirradiated cells.

Mutation frequency

An approach to human mutagenicity monitoring that makes use of cultured lymphocytes has been proposed and widely discussed [32-33]. The method is based on the autoradiographic detection of 6-thioguanine resistant ($6TG^R$) pheripheral blood lymphocytes arising *in vivo* and revealed in culture as cells capable to divide and to incorporate ^{3}H-TdR in medium containing the purine analog. Samples of 3 x 10^6 PHA-stimulated lymphocytes were incubated in the presence of 2.2 x 10^{-4} M 6TG for 24 h and labeled with 1 μCi/ml of ^{3}H-TdR (specific activity 2 Ci/mmol) for 6 h. Control cultures were treated in the same way except for the addition of 6TG. The frequency of labeled cells was calculated on autoradiographic slides by scoring 2500 nuclei in the control samples and about 10^6 nuclei in the 6TG-treated samples.

Genetic instability studies on selected samples of individuals

The following individuals at risk for environmental and/or genetic factors were analyzed: 1) individuals from the Seveso population exposed to TCCD; 2) psoriatic patients exposed to photochemotherapy; 3) members of a family with a high incidence of colonic cancer; 4) patients affected by nevoid basal cell carcinoma syndrome; 5) patients with photosensitivity.

Individuals from the Seveso population

A sample of the Seveso population exposed to TCDD during the accident at ICMESA plant (July 1976) [34] was re-examined after a three year interval [35]. The analysis was performed on 21 individuals that at the time of the first investigation showed an incidence of more than 6% of anomalous mitoses. The Seveso sample included 13 males and 8 females, aged 9 to 68 years; of these 17 were people exposed to the toxic cloud (acute exposure); 2 were workers at the ICMESA plant (chronic exposure) and 2 were normal subjects living in the surrounding uncontaminated area (control category). For each individual, the frequency of chromosomal aberrations and of SCE and UDS induced by UV irradiation were determined on the same blood sample.

The results of the cytogenetic analyses did not show any significant differences between the control group and the Seveso group for the average proportion of aberrant cells, both including and excluding gaps, and for the mean number of different types of anomalies per cell, at the two culture times (Table 1). Likewise no difference was found for SCE frequency; the mean number of SCE per cell was 10.45 ± 0.30 and 10.25 ± 0.47 in the Seveso and control samples respectively ($p < 0.60$), the ranges being 4.19-18.81 and 3.72-19.11.

The data on repair synthesis in G_0 lymphocytes are presented in Fig. 1 and in Table 2. The mean values of UV-stimulated ^{3}H-TdR incorporation in the presence of hu increase with incubation time and with UV dose in

Table 1. - *Average proportion (± SE) of abnormal cells and mean number (± SE) of chromosome aberrations per abnormal cell in 48 and 72 h cultures*

		Seveso sample *(a)*	Control sample *(b)*	p
48 h				
Aberrant cells (%)	Including gaps	3.81 ± 0.62	3.33 ± 1.39	> 0.50
	Excluding gaps	1.49 ± 0.31	0.86 ± 0.49	> 0.20
Type of aberration	cd gaps	2.05 ± 0.43	1.79 ± 0.91	> 0.50
	cm gaps	0.21 ± 0.10	0.41 ± 0.30	> 0.40
	cd breaks	0.75 ± 0.25	0.45 ± 0.34	> 0.50
	cm breaks	0.40 ± 0.13	0.31 ± 0.23	> 0.50
	Rearrangements	0.07 ± 0.05	0.02 ± 0.04	> 0.50
72 h				
Aberrant cells (%)	Including gaps	3.27 ± 0.41	4.04 ± 0.48	> 0.20
	Excluding gaps	1.06 ± 0.21	1.37 ± 0.41	> 0.40
Type of aberration	cd gaps	1.66 ± 0.28	2.42 ± 0.31	> 0.50
	cm gaps	0.31 ± 0.12	0.11 ± 0.07	> 0.10
	cd breaks	0.29 ± 0.12	0.25 ± 0.15	> 0.50
	cm breaks	0.54 ± 0.15	0.94 ± 0.28	> 0.10
	Rearrangements	0.01 ± 0.01	0.01 ± 0.01	> 0.50

(a) 21 individuals; *(b)* 7 and 15 individuals analyzed at 48 and 72 h respectively; cd = chromatid; cm = chromosome

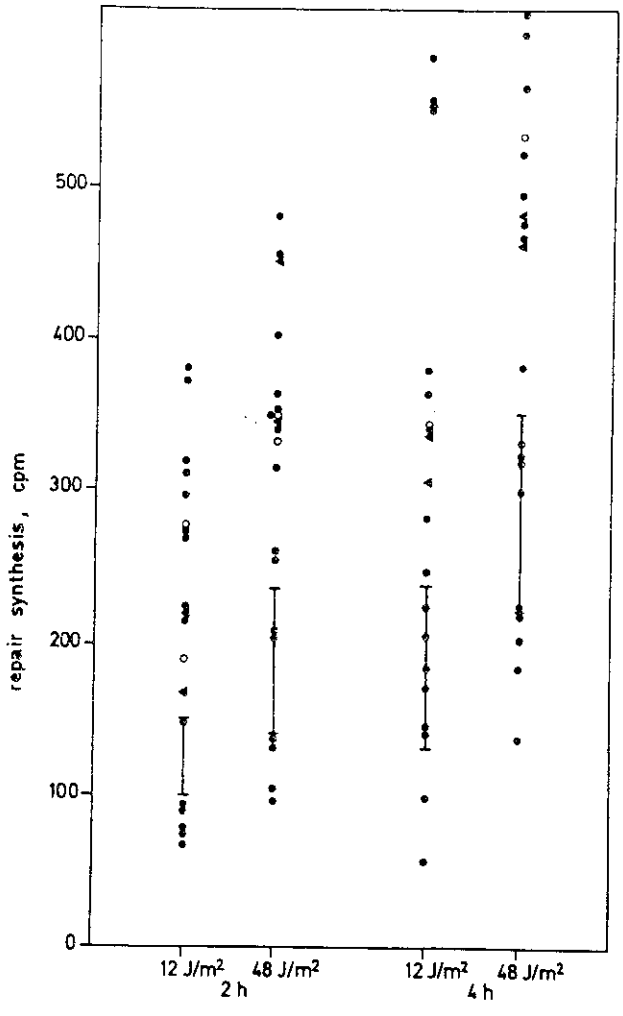

Fig. 1. - Repair synthesis values in G_0 lymphocytes from Seveso individuals (● = acute exposure; ◀ = chronic exposure; ○ = control category). The range of variability (mean value ± 95% confidence limits) in 16 healthy donors is reported.

the control sample. A similar trend is observed in the Seveso sample, although the values show a wider distribution. The mean values of UDS in the four experimental conditions are significantly higher in the Seveso sample than in the control. Since the same rate of DNA synthesis insensitive to hu was present in the two samples of individuals, the observed difference in UDS might be due to an abnormal responsiveness to UV irradiation in subjects selected from the Seveso population. However no relationship could be established between increased repair activity and exposure to TCDD.

Psoriatic patients

A group of psoriatic patients exposed to 8 MOP in combination with long-wave ultraviolet radiation (PUVA) was analyzed [36]. PUVA therapy is considered an effective treatment for psoriatic skin lesions; on the other hand experimental and clinical observations suggested that PUVA photochemotherapy may have mutagenic and tumor promoting effects.

Twenty-two patients aged 22-84 years were studied. Twelve of them were under therapy at the time of the investigation (PUVA patients); ten received the last treatment from 1 to 19 months before the investigation (ex-PUVA patients). The cumulative UVA dose received by each patient varied from 272 to 3709 J/m² during a period of 3-84 months. Twenty-two healthy donors were analyzed in parallel.

The sensitivity to mutagens, including 8 MOP plus UVA light, was measured in stimulated lymphocytes after treatment of G_0 cells. As shown in Fig. 2 the reduction of DNA synthesis rate in cells from the patients was similar to that observed in cells from healthy individuals, indicating that photochemotherapy does not significantly affect the response to DNA damaging agents. Similarly the frequency of mutant cells (cells capable to duplicate in medium containing 6TG), was in the normal range. The mean value of $6TG^R$ cells was 0.50×10^{-5} in five PUVA patients and 0.85×10^{-5} in eleven healthy individuals.

An abnormal feature that emerged from the investigation in this group of individuals was the reduced responsiveness to mitogen stimulation. The DNA duplication rate was lower both in PHA and PWM stimulated cells of PUVA patients, as demonstrated by ^{3}H-TdR incorporation values that were about one third of those in healthy individuals. DNA synthesis reduction was paralleled by a reduced proliferation kinetics; the frequency of cells undergoing two or more divisions was lower in cultures

Table 2. - *Mean values of repair synthesis (^{3}H-TdR cpm/3 x 10^5 cells ± SE) after irradiation with two UV doses, and after two incubation times*

UV dose	Incubation time	Seveso sample *(a)*	Control sample *(b)*	p
12 J/m²	2 h	213.2 ± 23.25	125.4 ± 11.82	< 0.005
	4 h	290.0 ± 35.27	184.9 ± 24.42	< 0.05
48 J/m²	2 h	296.5 ± 26.35	188.9 ± 22.01	< 0.005
	4 h	392.5 ± 33.40	287.4 ± 30.07	< 0.05

(a) 20 individuals; (b) 16 individuals

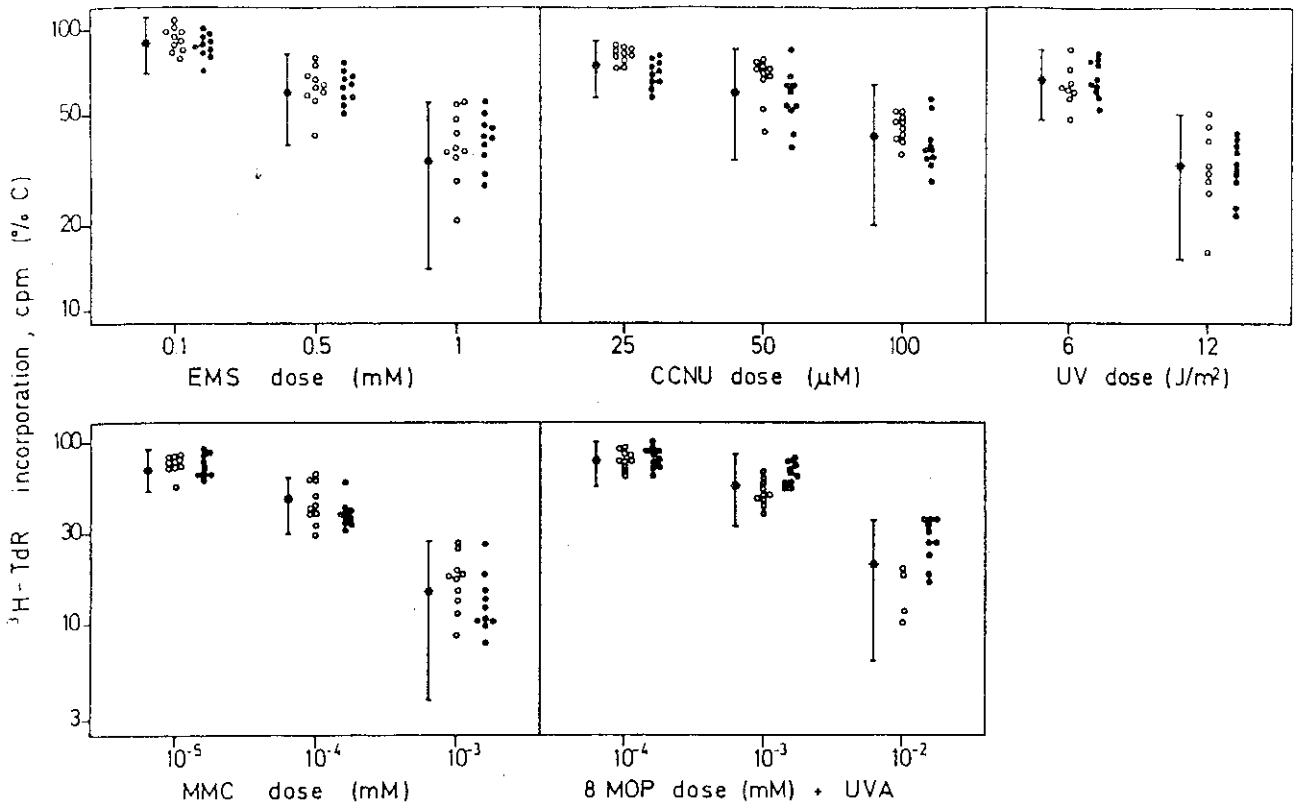

Fig. 2. - DNA synthesis rate in stimulated lymphocytes after exposure of G_0 cells to mutagens. Incorporation values of ^{3}H-TdR in mutagen expressed as percentage of the value in untreated control cells (c). The range of variability (mean value ± 95% confidence limits) in 22 healthy donors is reported. The symbols refer to ex PUVA (o) and PUVA (•) patients. Each point is the mean of the values in PHA and PWM stimulated samples.

from PUVA patients compared with cultures of healthy subjects (40% and 60% respectively).

A remarkable finding derived from the cytogenetic analysis: while a normal frequency of SCE (10.89 ± 0.89 in the patients, 9.33 ± 1.06 in the healthy donors) was observed, the rate of chromosomal breakage was significantly higher in patients under PUVA therapy compared to healthy individuals (Table 3).

This observation suggested that photochemotherapy may induce genetic damage, and indicates that the clastogenic effect should be monitored during PUVA treatment.

Members of a family with high incidence of colonic cancer

A large family with a high incidence of colonic cancer was investigated [37]. Six affected individuals (aged 39-56 years), seventeen relatives (35-62 years) and thirteen healthy donors were analyzed. Blood samples, divided in six different shipments, reached the laboratory 15-20 h after drawing. Cytogenetic analysis demonstrated that the frequency of mitoses with chromosome aberrations was in the normal range in all the members of the family with two exceptions: an affected individual (6.9% of mitoses with breaks, 3.4% with rearrangements) who was under chemotherapy at the time of examination and a healthy individual (9.10% of mitoses with breaks). This last finding remained unexplained.

The capacity to repair UV-induced DNA damage was evaluated by measuring UDS in G_0 lymphocytes. The values obtained with cells of the patients, their relatives and normal donors were not significantly different. However, in this group of experiments the variability in the response to UV irradiation among cells from normal donors was greater than that usually observed. This variability is probably attributable to non homogeneous conditions of blood sample storage during shipment.

The sensitivity to mutagens was investigated in the lymphocyte subpopulations stimulated with PHA and PWM. The exposure of G_0 cells to varying doses of UV light, mono and bifunctional alkylating agents was found to inhibit the DNA synthesis rate to the same extent in all the individuals analyzed.

The overall results of this study indicate that the predisposition to colonic cancer in the investigated family does not correspond to genetic instability detectable with the assay systems used.

Patients affected by nevoid basal cell carcinoma syndrome (NBCCS)

A small sample (four subjects) of patients affected by NBCCS was investigated to establish whether the occur-

Table 3. - *Average proportion (± SE) of abnormal mitoses in psoriatic patients exposed to photochemotherapy*

	Aberrant cells (%)		
	Including gaps	Excluding gaps	With rearrangements
Healthy controls (14)	3.07 ± 0.74	1.41 ± 0.56	0.087 ± 0.087
Patients ex PUVA (9)	4.03 ± 0.86 0.40 > p > 0.3	2.04 ± 0.59 0.4 > p > 0.3	0.23 ± 0.15 0.4 > p > 0.3
PUVA (12)	4.98 ± 0.92 0.2 > p > 0.1	2.75 ± 0.51 0.05 > p > 0.02	0.45 ± 0.16 0.05 > p > 0.02

Number of analyzed individuals in parenthesis.

rence of basal cell tumors observed in some patients after radiotherapy corresponds to cellular manifestations of genetic instability [38]. Cytogenetic analysis revealed an increase both in the percentage of abnormal mitoses and in the mean number of breaks per cell in NBCCS patients compared with control individuals; however the difference was not statistically significant and too low to be effectively used as a cellular marker of the disease.

The level of UDS in G_0 lymphocytes following UV exposure, and the DNA synthesis rate in stimulated lymphocytes following treatment with different DNA damaging agents (EMS, MMC, CCNU, PUVA, UV) were within the normal range. Also the tendency to mutability, analyzed by measuring the frequency of $6TG^R$ cells in lymphocyte cultures, was found similar to that observed in normal individuals. These findings indicate that the chromosomal instability and cellular UV hypersensitivity described in some NBCCS patients are not distinctive and constant NBCCS features.

Patients with photosensitivity

We report here results of investigations performed on eight patients referred to us by pediatric or dermatologic clinics. Five patients (XP5, XP6, XP7, XP8, XP9), with presumptive diagnosis of xeroderma pigmentosum, presented various degrees of cutaneous alterations in photoexposed areas. Three patients (TTD1, TTD2, TTD3) were affected by trichothiodystrophy, a rare hereditary disorder characterized by brittle hair, mental and physical retardation; they showed also severe photosensitivity.

Chromosome analysis did not reveal chromosome fragility or abnormal frequency of SCE. However in lymphocyte cultures of the XP9 patient despite the normal frequency of mitoses with gaps and breaks (1.3%), the frequency of mitoses with chromosome rearrangements (3.4%) was higher than that commonly found in healthy individuals. Subsequent cytogenetic analyses in fibroblast cultures allowed the identification of four mutant clones, each characterized by a specific translocation. The rearranged chromosomes were n. 2, 13, 14, 15 [39].

In stimulated lymphocytes from all the patients the sensitivity to EMS, MMC, CCNU was in the normal range. On the contrary, hypersensitivity to UVC irradiation leading to a marked reduction of the DNA duplicative rate was found. After an UV dose of 7 J/m^2, cells from TTD patients showed ^{3}H-TdR incorporation values reduced by more than 90% of those in unirradiated cells and similar to those observed in XP cells; in the same experimental conditions the reduction in parent and normal subject cells was about 10%.

The analysis of UDS in G_0 lymphocytes demonstrated a defect in the repair synthesis of UV-induced damage. As shown in Fig. 3, the parents of affected individuals had UDS levels in the range of healthy donors; in contrast a 90-50% reduction of UDS was present in the patients. The residual level of UDS found in XP6, higher than that present in the affected brother reflects a very peculiar situation and has been attributed (among other possible explanations) to a mosaic condition for defective and normal revertant cells [40].

The defect conferring UV sensitivity was demonstrated also in fibroblasts cultured *in vitro* from the five XP and the three TTD cases. The genetic study was performed by complementation analysis of the UDS defect in hybrid cells obtained by fusion between cells from our patients and XP cells belonging to different complementation groups (c.g.). Results of these experiments indicated that XP5 and XP9 belong to c.g. C, XP6 and XP7 to c.g. A, TTD1, TTD2 and TTD3 to c.g. D.

The association of XPD and TTD mutations was subsequently confirmed by complementation study performed in fibroblasts from six other photosensitive TTD patients [41-43].

Besides the ascertainment of XP diagnosis in patients with severe cutaneous lesions, a particularly relevant result of this study was the finding of impairment to repair the UV-induced damage in patients with clinical diagnosis of TTD. Although they show photosensitivity causing sunburn after very short exposure to sunlight, they have not so far developed tumors or precancerous skin lesions typical of XPD individuals. Thus, detection

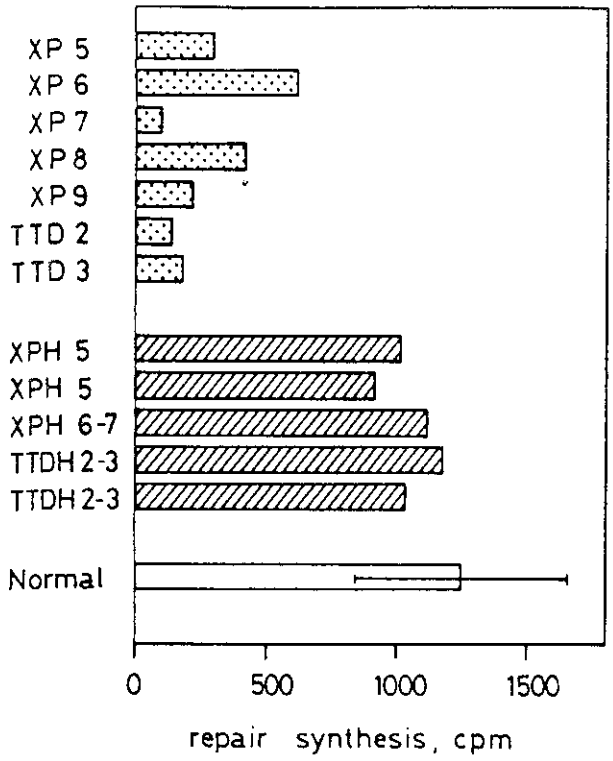

Fig. 3. - UV-induced DNA repair synthesis in G_0 lymphocytes from healthy individuals (normal), xeroderma pigmentosum (XP) and trichothiodystrophy (TTD) patients and their parents (XPH and TTDH).

of XPD mutation in these patients has a great importance for tumor prevention and therapy, and offers new insight for elucidating the correlation between DNA repair impairment and tumor proneness.

Concluding remarks

Cytogenetic analysis is the most extensively employed method to assess a genotoxic effect in mutagen exposed persons and to reveal genetic instability in subjects carrying cancer predisposing genes.

Chromosome damage is certainly a significant endpoint in the monitoring of genetic risk while the underlying causes and the relationships of both chromosome breakage and SCE with DNA repair defects and mutability are not defined.

As to the relation between chromosome rearrangements and neoplastic transformation, the study of preferential localization of chromosome lesions in subjects at risk and the contribution of fragile sites to non-random distribution of chromosome lesions may be of great interest.

UDS analyzed in mutagen challenged G_0 lymphocytes is a sensitive indicator of repair capacity. Patients carrying XP mutations of any complementation group are easily identified since UV-irradiated cells show at least 50% reduction of UDS value compared with cells from the parents and normal control individuals. However in healthy subjects interindividual variability can be found, also when blood samples are analyzed in parallel under standardized experimental conditions. At present the physiologic, pathologic and/or genetic factors that presumably influence this variability, are not known. More studies on more "healthy" donors as well as on repeated blood samples from the same donor are necessary to identify the UDS variability which may be generated by genetic instability.

Although the same limitations hold true in the evaluation of mutagen sensitivity by the measurement of the DNA duplication rate in stimulated lymphocytes, this assay appears particularly useful in a preliminary screening. In fact the test, being performed on a relatively low amount of cells, can be applied for analyzing in parallel mutagens which induce DNA lesions restored by different repair mechanisms. Furthermore this method allows the detection of mutagen hypersensitivity not necessarily related to reduced UDS activity. This is the case of Cockayne syndrome in which hypersensitivity to UV light is accompanied by normal UDS level.

It is worth noticing that in the XP patients studied by us a correlation was always found between UDS in G_0 cells and DNA duplicative synthesis in stimulated lymphocytes after UV exposure of G_0 cells.

According to our experience the use of stimulated cells gives more reproducible results in comparison with UDS analysis in G_0 cells particularly when blood samples cannot be processed immediately after drawing.

The mutagenicity test, the most direct method to evaluate genetic instability and its interaction with environmental genotoxic agents, is not yet extensively used; furthermore the available literature data are often conflicting mainly because experimental conditions to eliminate phenocopies have still to be worked out.

Results obtained in our laboratory on the frequency of 6TG resistant cells in small samples of individuals did not allow us to draw any conclusion owing lack of positive control subjects. It is necessary to establish more efficient methods for detecting and enumerating mutant lymphocytes, possibly extending the analysis to a panel of genetic markers.

Ideally all the discussed tests (cytogenetic analysis, sensitivity to mutagens, unscheduled DNA synthesis, mutability) should be included in the experimental protocol on the same blood sample for the identification of at risk subjects. The obtained information should be utilized not only to predict individual susceptibility to genotoxic damage but also to increase our knowledge on human genomic stability.

Acknowledgements

This work was partially supported by grants from Regione Lombardia (Ricerca Finalizzata Area Prevenzione) and from National Research Council of Italy (P.S. Mutagenesi). The authors thank Mrs D. Tavarné for typing the manuscript.

Review submitted on invitation by the Editorial Board of the *Annali*. Accepted for publication: October 1988.

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