PREMATURE CENTROMERE DIVISION AS A BIOMARKER OF GENOTOXIC INFLUENCES.

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Abstract
The analysis of spontaneous and UV-induced premature centromere division (PCD) in the peripheral blood lymphocytes of humans was carried out. We have estimated the level of metaphases with PCD (MPCD), rate of PCD and individual spectrum of chromosomes with PCD. The analysis of spontaneous level of PCD revealed hight inter-individual variability of MPCD level (from 0 to 6.00%, mean 1,85±0,45%), PCD rate (from 0% to 15.53%, mean 3.37±1.07%) and of spectra of chromosomes with PCD. After the ultraviolet irradiation of donor blood it was detected significant increasing of means of MPRC (3.16±0.77%, $p<0,01$), PCD (12.52±4,30%, $p<0,01$) and changes in spectrum of PCD-chromosomes. Our results suggest that increased PCD levels may indicate the effect of genotoxic exposures, and PCD can be a useful sensitive cytogenetic biomarker for detection of genotoxic influences.

Introduction.
Premature centromere division (PCD) is the early separation of one or several chromosomes in centromere regions during prometaphase or metaphase while the rest of them have distinctive X- or V-like morphology. The published data suggest that PCD are not incidentals [1] and may be possible manifistation of chromosomal instability [2]. PCD has been investigated by few reseach groups in relationship with Roberts' syndrome of chromosomal instability [1] and neoplasias (especially gematological) [3].

It is suggested that PCD reflects some problems at the spindle checkpoint that lead to chromosome loss (gain) during mitosis [4]. Chromosome losses, especially after genotoxic exposures, are themajor initial cancer events [2]. In spite of that, PCD is not regarded as an obligatory parameter of cytogenetic analysis. Only few published investigations are devoted to PCD as biomarker of genotocsic exposures and cancer risk assessment [5]. Thus the question about applicability of PCD test for monitoring studies in human populations exposed to genotoxic agents remains open.

In this study we investigated the informative valuability of PCD as biomarker of genotoxic influences. We present data on spontaneous and ultraviolet induced levels of PCD in human lymphocytes; quantitative analysis of chromosome classes involved in PCD, and tried to define individual's features of spontaneous and induced PCD.

Methods.
Blood from 15 persons (males of age 30-50) was collected by venepuncture. All donors passed medical examination and were declared clinically healthy. The group of donors included neither heavy smokers (>20 cigarettes/day) nor heavy drinkers (>100 ml of strong alcohol drinks a day). None of the donors had ocupational genotoxic exposure.

Samples of whole blood (1 ml) were cultivated in 5 ml of IMDM medium. PHA-P ("GENOME", Donetsk) (5 μg per 1 ml of medium) was used for blasttransformation of lymphocytes. Cultures were incubated at 37 °C for 52 h. Before the cultivation, part of blood samples (1mm layer) was irradiated with ultraviolet (UV-C) (a BUV-15 lamp, a UVS-1 filter) for 1 min, which corresponds to 103.2 J/m^2. The UV dose was selected according to results of our previous investigations [6].

Colchicine (1 μg/ml, final concentration) was added at 1.5 - 2 hours before the end of cultivation. After

cultivation cells were treated with 0.075 M KCl and fixed in methanol : acetic acid (3:1) mixture.

Metaphase plates were prepared by dropping of cell suspension onto cool moist slides. Slides were stained by routine Giemsa technique according to standard procedures [7].

The slides were scored for frequency of metaphase plates with PCD (MPCD), and for the level of PCD (the number of PCD-chromosomes per 100 metaphases). Chromosome classes (A-G) involved in PCD were scored, also. PCD was diagnosed when a separation between the sister chromatides was equal to or more than thickness of chromatide. Statistical significance of differences was estimated by the 2×2 chi-square (χ^2) test [8].

Results.
The metaphases with PCD (Fig. 1) were found on the slides of almost all donors (13 persons from 15). Usually one or two chromosomes of the chromosome set were involved in PCD.

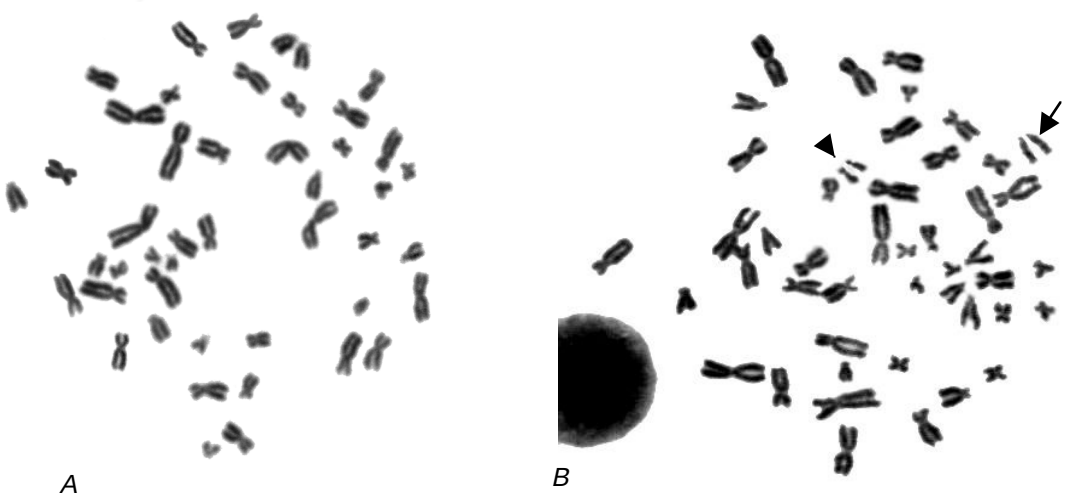

Fig. 1 The phenomen of premature centromere division in culture of perepheral blood lymphocytes of human: *A* - normal metaphase plate; *B* - premature centromere division of chromosomes of groupes D (short arrow) and E (long arrow)

The data for the levels of MPCD and PCD in lymphocytes depending on the UV-irradiation are presented in the Table 1. Spontaneous frequency of MPCD varied from 0% to 6.00±2.37% with mean 1.85±0.45%. Frequency of PCD for these samples varied from 0% to 15.43±2.05%, average level was 3.37±1.07%. The highest levels of PCD were detected for chromosome classes C (30±4.80%), A (24±4.45%) and E (24±4,45%). Chromosomes of classes D, F and G involved in PCD in a lesser degree (3.30±1.85%, 5.40±2.36%, 5.40±2.36% respectively).

After UV-C irradiation the average levels of MPCD and PCD significantly increased. Mean for MPCD increased from 1.85±0.45% without irradiation to 3.16±0.77% after irradiation (p<0.01), and for PCD – from 3.37±1.07% without irradiation to 12.52±4.30% after UV irradiation (p<0.01). The level of PCD increased more than åPCD (Table 1). It may suggest of existence of a subpopulation of cells with defects of chromosome segregation. Then mutagenic influences increase the expression of PCD mainly in these cells.

UV irradiation induced alterations in spectrum of groups of PCD-chromosomes (Fig. 2). Against the background of total increasing of PCD frequency the fraction of chromosomes of A group involved in PCD decreased from 24 ± 4.45% without irradiation to 14 ± 2.51% after UV irradiation (p < 0.05). The contribution to total PCD level for chromosomes of C group increased from 30 ± 4.80% without irradiation to 44±3.55% after irradiation (p < 0.05).

Table 1. Individual levels of MPCD and PCD

N of donor	Non-irradiated cultures		UV-irradiated cultures	
	MPCD, %	PCD, %	MPCD, %	PCD, %
1	0.99±0.70	2.48±1.09	2.00±1.40	7.00±2.55
2	6.00±2.37	10.00±3.00	10.00±3.00	50.00±5.00
3	1.00±0.99	1.00±0.99	3.00±1.71	6.00±2.37
4	0.47±0.46	1.40±0.80	0	0
5	2.00±1.40	4.00±1.96	4.46±1.65	12.74±2.67
6	2.00±1.40	2.00±1.40	8.00±2.71	51.00±5.00
7	1.58±0.72	2.77±1.03	5.00±2.18	22.00±4.14
8	5.14±1.25	15.43±2.05	5.00±2.18	9.00±2.86
9	0.50±0.50	0.50±0.50	1.00±0.99	1.00±0.99
10	0	0	1.00±0.99	5.00±2.18
11	2.00±1.40	3.00±1.71	4.00±1.96	5.00±2.18
12	3.00±1.71	3.00±1.71	3.00±1.71	16.00±3.67
13	1.00±0.99	1.00±0.99	0	0
14	0	0	0	0
15	3.00±1.40	4.00±1.96	1.00±0.99	3.00±1.71
Mean	1.85±0.45	3.37±1.07	3.16±0.77**	12.52±4.30**

** - $p<0.01$

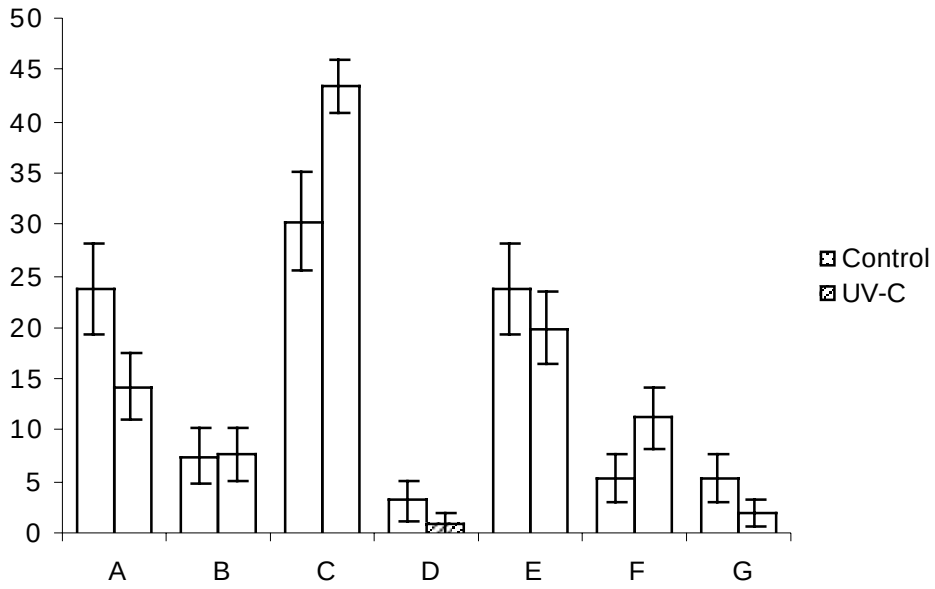

Fig. 2 Alteration of chromosomal spectrum of PCD after UV-irradiation. A-G – classes of chromosomes.

In the table 2 individual the group spectra of PCD-chromosomes are presented. The analysis of the results revealed high inter-individual variability of PCD rates as well as of individual spectra of chromosomes involved in PCD. The comparison of spontaneous and UV-induced PCD suggests obvious inter-individual differences in response to mutagenic influences. It is clear manifested both for the total level of PCD and for individual chromosomal spectra of PCD.

Table 2. Individual's spectra of chromosomes involved in PCD.

N of donor	Non-irradiated cultures							UV-irradiated cultures						
	A	B	C	D	E	F	G	A	B	C	D	E	F	G
1	0.98			0.5	0.5		0.5	1		4		2		
2	3		5		2			8	3	21	2	9	6	1
3			1							3		1	1	1
4	0.93				0.47									
5	2	1		1				1.27	0.64	7.65		1.91	1.27	
6				1	1			11	4	18		9	7	2
7	1.19		1.58					3	2	11		5	1	
8	3.21	1.61	4.5		3.86	1.29	0.96		2	3		3	1	
9					0.5							1		
10								1	1	2		1		
11		1	1		1					1		2	2	
12			3					1	2	8		3	2	
13					1									
14														
15					2	1	1	1		2				

A-G – classes of chromosomes. Frequency of PCD is presented in percents.

Discussion

Regulation of and segregation during mitosis is under strong genetic control. Defects in mechanisms that normally maintain the fidelity of these processes can lead to aneuplidy [2]. The spindle checkpoint mechanism plays a key role in ensuring in time chromatide separation and segregation. They monitor the proper interaction between chromosomes and microtubules, and delays metaphase to anaphase progression (forbid sister chromatide separation) if one or several kinetochores not attached to mitotic spindle [4].

The standard procedures of chromosome slide preparation use mitotic poison of colchicine or colcemide to arrest cells at metaphase stage [7]. None of the chromosomes are attached to micritubules and spindle checkpoint are switched on. In this case, observed early separation of one or several chromosomes (premature centromere division (PCD)) may reflects alterations of proper processes of the spindle checkpoint. Thus PCD may be used as parameter of chromosome instability.

Our data suggest that PCD is a sensitive end-point of genotocsic influence. Irradiation of UV-C, as model mutagene, significantly increases levels of MPCD and PCD. The level of PCD increased significantly more then MPCD. It may suggest of existence of a subpopulation of cells defected by mechanisms of chromosome segregation. Thus, a mutagenic influence intensifies an expression of PCD mainly in these cells.

Alteration of chromosomal spectrum after UV-exposure suggest about differences in sensitivity of various chromosomal classes to mutagenic action. It may depend on particularities of centromeric architecture of different chromosomes.

Our data suggest that spontaneous and induced levels of PCD as wall as spectrum of chromosomes involved in PCD may depend on donor genotype. It is adjust with opinion of R Madan et al [9] that PCD is inherited cytogenetic anomaly. The analysis of the individual chromosomal spectra of PCD allow obtain information about personal features of PCD expression. More detailed analysis of

individual spectra by chromosomes with PCD (especially chromosomes with known tumor suppressor genes) may be useful as method for individual cancer risk assessment.

Conclusions

1. PCD is a sensitive marker of genotoxic influences.
2. The premature centromere division may be described with three parameters: frequency of MPCD, PCD level and spectrum of chromosomes involved in PCD. Parameters are not independent but they let to get additional information on defects of processes of normal segregation of chromosomes.
3. The particularities of spontaneous and UV-induced manifestations of PCD allow detecting of individual's features of PCD expression.

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