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INDUCTION OF CHROMOSOMAL ABERRATIONS IN HUMAN CELLS BY A TEMPERATURE-SENSITIVE MUTANT OF HERPES SIMPLEX VIRUS TYPE 2 AND ITS REVERTANTS

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Summary. — The induction of chromosomal aberrations by a temperature-sensitive (ts) mutant of herpes simplex virus type 2 (HSV-2) strain Hg52 (ts 13), its revertants 4—8 and 5—8 and by etalon strains HSV-1 17 syn$^+$ and HSV-2 Hg52 was studied in human fibroblast and lymphocyte cultures. The effect on chromosomes of the revertants was tested at permissive (31 °C) and non-permissive (38 °C) temperatures. At 38 °C the revertants could not induce DNase activity. The present results contribute to the possible role of a herpes-coded nuclease in induction of chromosomal aberrations.

Key words: herpes simplex virus; mutants; human chromosomes; aberrations

The induction of chromosomal aberrations by herpes simplex viruses (HSV) in mammalian cells was well established (Hampar and Ellison, 1963; Stich *et al.*, 1964). It is likely that chromosomal changes occur due to the action of virus-coded early enzymes (Zur Hausen, 1967, 1968; Waubke *et al.*, 1968), such as exo- and endonucleases (Morrison and Keir, 1968; Hoffman and Cheng, 1979) and an herpes-specific DNA polymerase, possessing exonuclease activity (Knopf, 1979). It was of interest to know the efficiency of HSV-2 ts13 revertants to induce chromosomal aberrations at 38 °C a temperature at which they could not exert any DNase activity.

Human diploid embryonic lung fibroblasts (HL) were used in the 8th or 10th passages. The cultures met the international standard requirements for diploid cell strain. The fibroblast and lymphocyte cultures were grown, infected and examined as described (Mincheva *et al.*, 1984). HSV-1 strain 17syn$^+$, HSV-2 strain Hg52, ts mutant HSV-2 ts 13 and its revertants 4—8 and 5—8 were generously provided by J. H. Subak-Sharpe (Glasgow collection). The multiplicity of infection varied from 1.5 to 1.7. The effect of infection on the chromosomes was tested at 6 and 10 hr post-infection (p.i.). One of the characteristics of HSV-2 ts 13 is its inability to induce alkaline DNase activity at 38 °C. Despite the high efficiency of plaquing at 38 °C of

Table 1. Chromosomal aberrations in HSV-infected HL cells at 31 °C

Aberration	HSV strains					
	17syn*	Hg52	ts13	ts13rev (4—8)	ts13rev (5—8)	Control
Chromatid breaks	48*	7	1	8	7	—
Chromosome breaks	5	2	—	1	—	—
Gaps	30	16	10	13	14	5
Endoreduplication	2	—	—	—	—	—
Pulverization	15	—	—	—	—	—
Despiralization	3	—	—	—	—	—
Tetraploidy	5	1	—	—	2	—
Metaphases with severe chromosome damage***	10	—	—	—	—	—
Highly micronucleated cells	2	—	—	—	—	—
Cells with more than one aberration	16	5	—	—	—	—
Total	120 (74.5%)	30 (25.2%)	11 (10.4%)	22 (20.5%)	23 (18.1%)	5 (3.8%)
Number of cells examined	161	119	105	107	127	130
P(t)	$p < 0.001$					
Chromatid breaks	8**	12	3	23	11	—
Chromosome breaks	2	2	3	3	—	—
Gaps	16	10	11	6	8	4
Endoreduplication	—	—	—	—	—	—
Pulverization	13	15	—	—	—	—
Despiralization	2	—	—	—	—	—
Tetraploidy	—	—	2	—	1	—
Exchanges	1	—	1	—	—	—
Metaphases with severe chromosome damage	—	—	—	6	3	—
Highly micronucleated cells	7	—	—	—	—	—
Cells with more than one aberration	12	5	2	5	3	—
Total	49 (43.3%)	39 (37.8%)	20 (17.0%)	38 (27.3%)	23 (22.1%)	4 (3.3%)
Number of cells examined	113	103	117	139	104	120
P(t)	$p < 0.001$					

* Number of aberrations at 6 hr p.i. (out of total).
** Number of aberrations at 10 hr p.i. (out of total).
*** Multiple chromatid and chromosome breaks and gaps.

ts 13 revertants 4—8 and 5—8, production of DNase activity was as impaired as with the 13 HSV-2 mutant (Moss *et al.*, 1979).

The number and the type of chromosomal aberrations in both fibroblast and lymphocyte cultures at 6 and 10 hr p.i., cultivated at 31 and 38 °C are

Table 2. Chromosomal aberrations in HSV-infected lymphocytes at 31 °C

Aberration	HSV strains					
	17syn*	Hg52	ts13	ts13rev (4–8)	ts13rev (5–8)	Control
Chromatid breaks	9*	14	12	18	20	—
Chromosome breaks	2	1	—	3	1	—
Gaps	57	38	21	36	30	2
Endoreduplication	1	—	—	—	—	—
Pulverization	—	—	—	—	—	—
Despiralization	—	—	—	—	—	—
Tetrapliidy	1	—	—	1	—	—
Metaphase with severe chrom. damage***	8	1	—	—	—	—
Highly micronucleated cells	—	—	—	—	—	—
Cells with more than one aberration	15	7	5	12	12	—
Total	78 (64.4%)	54 (49.0%)	33 (32.3%)	58 (45.3%)	51 (43.2%)	2 (1.8%)
Number of cells examined	121	110	102	128	118	110
P(t)	$p < 0.001$					
Chromatid breaks	5**	4	6	9	8	—
Chromosome breaks	2	—	—	2	2	—
Gaps	28	27	24	21	14	3
Tetraploidy	1	—	—	—	—	—
Exchange	—	1	—	1	—	—
Acentric phragment	—	—	1	—	—	—
Cells with more than one aberration	7	4	5	5	2	—
Total	36 (33.9%)	32 (29.6%)	31 (27.6%)	33 (26.1%)	24 (21.0%)	3 (2.5%)
Number of cells examined	106	108	112	126	114	116
P(t)	$p < 0.001$					

* Number of aberrations at 6 hr p.i. (out of total).
** Number of aberrations at 10 hr p.i. (out of total).
*** Multiple chromatid and chromosome breaks and gaps.

shown in Tables 1 and 2, and in Tables 3 and 4, respectively. Our results revealed typical karyological effects of the examined HSV strains (Mincheva *et al.*, 1984), which were shown to depend on the serotype used as well as on the kind of cells examined. A significant difference in the occurrence of metaphases with aberrations was visible with revertants at 31 °C and at 38 °C. In contrast to the different chromosomal lesions at 31 °C, a significant decrease of the chromosomal aberrations in both infected cells with the revertants was noted at 38 °C. In addition, the results also support the probability of the action of a virus-coded nuclease for development of HSV-in-

Table 3. Chromosomal aberrations in HSV-infected HL cells at 38 °C

Aberration	HSV strains		
	ts13rev(4−8)	ts13rev(5−8)	Control
Chromatid breaks	3*	5	—
Chromosome breaks	—	—	—
Gaps	10	11	4
Metaphases with severe chromosome damage	—	2	—
Cells with more than one aberration	—	—	—
Total	13 (11.8%)	18 (16.8%)	4 (3.6%)
Number of cells examined	110	107	110
P(t)	$p < 0.001$		
Chromatid breaks	5**	3	—
Chromosome breaks	1	2	—
Gaps	12	5	3
Endoreduplication	1	—	—
Tetraploidy	1	—	—
Total	20 (15.3%)	10 (9.5%)	3 (2.5%)
Number of cells examined	130	105	120
P(t)	$p < 0.001$		

* Number of aberrations at 6 hr p.i. (out of total).
** Number of aberrations at 10 hr p.i. (out of total).

duced chromosome aberrations. The observed low incidence of chromosomal lesions at 38 °C might be due to the very low levels of the enzyme in revertant-infected cells (Moss *et al.*, 1979) as well as due to the action of other virus-coded enzymes.

The distribution of aberrations along the length of each chromosome was non-random and was not proportional to the chromosome length (Table 5). There was a statistically significant difference between observed and expected distributions. As common action of the HSV strains was the preferential damage of chromosomes 1 and 3 followed by group B as previously described (Mincheva *et al.*, 1984).

The localization of the most frequently observed aberrations (Mincheva *et al.*, 1984) was the same in either cell type. There was no difference in the sites of damages of each chromosome damage after infection with the etalon strains and mutants. Beside the early localized aberrations in chromosome 1, additional damages were also observed, namely in the short arm — Ip22 and in the long arm — Iq25 and Iq42, which extend the regions of the selective action of HSV on human chromosome.

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Table 4. Chromosomal aberrations in HSV-infected lymphocytes at 38 °C

Aberration	HSV strains		
	ts13rev(4−8)	ts13rev(5−8)	Control
Chromatid breaks	3*	1	—
Chromosome breaks	—	—	—
Gaps	6	8	2
Total	9	9	2
	(8.8%)	(7.6%)	(1.1%)
Number of cells examined	102	118	120
P(t)	$p < 0.001$		
Chromatid breaks	2**	4	—
Chromosome breaks	3	—	—
Gaps	10	8	3
Tetraploidy	1	—	—
Metaphase with severe chrom. damage	—	1	—
Total	16	13	3
	(12.8%)	(10%)	(2.5%)
Number of cells examined	125	130	118
P(t)	$p < 0.001$		

* Number of aberrations at 6 hr p.i. (out of total).
** Number of aberrations at 10 hr p.i. (out of total).

Table 5. Distribution of breaks and gaps along the length of chromosomes of cultured human lymphocytes after infection with HSV strain ts13rev(4—8)

Chromosome No.	Number of aberrations		Diference	$P(\chi^2)$
	observed	expected		
1	11	4.90	+6.10	$< 0.01 > 0.001$
2	3	4.59	−1.59	> 0.05
3	11	3.85	+7.15	< 0.001
4	9	6.86	+2.14	> 0.05
5				
6−12, XX	14	21.82	−7.82	$< 0.05 > 0.01$
13−15	8	5.68	+2.32	> 0.05
16−18	1	2.32	−1.32	> 0.05
19−20	0	2.56	−2.56	
21−22	0	1.82	−1.82	
Total	57	57.00	0	

References

Hampar, B., and Ellison, S. (1963): Cellular alterations in the MCH line of Chinese hamster cells following infection with herpes simplex virus. *Proc. natn. Acad. Sci. U.S.A.* **49**, 474—480.

Hoffmann, P., and Cheng, V.-C. (1979): DNase induced after infection of KB cells by herpes simplex virus type 1 or type 2. II. Characterization of an associated endonuclease activity. *J. Virol.* **32**, 449—457.

Knopf, K. (1979): Properties of herpes simplex virus DNA polymerase and characterization of its associated exonuclease activity. *Eur. J. Biochem.* **98**, 231—235.

Mincheva, A., Dundarov, S., and Bradvarova, I. (1984): Effects of herpes simplex virus strains on human fibroblast and lymphocyte chromosomes and the localization of chromosomal aberrations. *Acta virol.* **28**, 97—106.

Morrison, J., and Keir, H. (1968): A new DNA-exonuclease in cells infected with herpes virus: Partial purification and properties of the enzyme. *J. gen. Virol.* **3**, 337—347.

Moss, H., Chartrand, P., Timbury, M., and Hay, J. (1979): Mutant of herpes simplex virus type 2 with temperature-sensitive lesions affecting virion thermostability and DNase activity: Identification of the lethal mutation and physical mapping of the nuc$^-$ lesion. *J. Virol.* **32**, 140—146.

Stich, H., Hsu, T., and Rapp, F. (1964): Viruses and mammalian chromosomes. I. Localization of chromosome aberrations after infection with herpes simplex virus. *Virology* **22**, 349—445.

Waubke, R., Hausen, H., and Henle, W. (1968): Chromosomal and autoradiographic studies of cells infected with herpes simplex virus. *J. Virol.* **2**, 1047—1054.

Zur Hausen, H. (1967): Induction of specific chromosomal aberrations by adenovirus type 12 in human embryonic kidney cells. *J. Virol.* **1**, 1174—1185.

Zur Hausen, H. (1968): Association of adenovirus type 12 deoxyribonucleic acid with host cell chromosomes. *J. Virol.* **2**, 218—223.