ISSN 1990-519X, Cell and Tissue Biology, 2015, Vol. 9, No. 2, pp. 119–126. © Pleiades Publishing, Ltd., 2015.
Original Russian Text © T.M. Grinchuk, M.A. Shilina, L.L. Alekseenko, 2014, published in Tsitologiya, 2014, Vol. 56, No. 11, pp. 841–849.

Long-Term Cultivation of Chinese Hamster Fibroblasts V-79 RJK under Elevated Temperature Results in Karyotype Destabilization

T. M. Grinchuk*, M. A. Shilina, and L. L. Alekseenko

Institute of Cytology, Russian Academy of Sciences, St. Petersburg, Russia

**e-mail: grintat@bk.ru*

Received August 15, 2014

Abstract—In this article, we show that long-term cultivation of Chinese hamster fibroblasts V-79 RJK at elevated temperature resulted in the selection of variants with genetic changes at the level of karyotype. Beginning at the first steps of thermoresistance (to a temperature of 40°C) selection, we identified a population of cells with changes in the karyotype (polyploidy, deletions, inversions, chromosomal translocations, cells with DM-chromosomes). Further cultivation was accompanied with selection of cells with breaks near centromeres and homogeneously staining regions on chromosomes. Nonspecific destabilization of the karyotype (at the initial stages of selection) was accompanied with increased gene expression of *hsc70* (constitutive isoform of heat shock protein of the HSP70 family) and *pgp1* (p-glycoprotein membrane transporter). Expression of these genes returned to the basal level during long-term cultivation at the elevated temperature, but the cells retained karyotypic changes.

Keywords: heat shock proteins, hyperthermia, karyotype destabilization, stress, temperature resistance

DOI: 10.1134/S1990519X15020078

INTRODUCTION

Genomic instability is a key problem of cell biology. Damage to DNA modifies the sister chromatide structure, resulting in altered chromosome structure (Morgan et al., 1996). Karyotypic instability is induced by various stressors, the most studied of which are ionizing radiation and chemical agents (Limoli et al., 1997).

A number of studies have been focused on the effect of hyperthermia on the genetic apparatus. The temperature that is optimal for an organism's vital activity is different in different organisms, but the defense mechanism called "heat shock response" is conservative.An increase in temperature induces denaturation and aggregation of protein structures, resulting in cellular disorders: modified cytoskeleton (Toivola et al., 2010), fragmentation of endoplasmic reticulum and Golgi complex and reduced mitochondria and lysosome number (Welch and Suhan, 1985), and disturbed RNA splicing (Vogel et al., 1995; Boulon et al., 2010). Multiple instances of damage and reorganization of cellular components reduce cell viability and may induce cell death. An organism's response to heat shock depends on the intensity and duration of hyperthermia, as well as on cell type. Moderate heating induces apoptosis or stress-induced cell senescence, while severe heat shock produces necrosis (Harmon et al., 1990; van der Waal et al., 1997; Alekseenko et al., 2012). A cell's response to hyperthermia is accompanied with induction and accumulation of heat shock proteins (Hsp), which are very conservative (Welch et al., 1991; Richter et al., 2010). Hsp are divided into two classes according to molecular weight. The first class includes Hsp with molecular weight of less than 40 kDa: Hsp32, Hsp27, and Hsp20 (Kregel, 2002). The other class includes proteins with higher molecular weight (60–100 kDa): Hsp60, Hsp70, Hsp90, and Hsp110 (Hildebrandt et al., 2002). Heat shock enhances the expression of both Hsp and other proteins (more than 100) that engage in protein folding, degradation, and transport, as well as DNA and RNA repair (Gasch et al., 2000; Tabuchi et al., 2008; Richter et al., 2010).

The cell reaction to hyperthermia depends on the cell cycle. Cells in S-phase and mitosis are more temperature-sensitive than cells in G_1 or G_2 phases (Westra and Dewey, 1971; Palzer and Heidelberger, 1973; Bhuyan et al., 1977). In S-phase, hyperthermia produces unrepaired DNA single-strand breaks. DNA double-strand breaks are evoked by heat shock at the border G_0/G_1 and G_2/M phases. Hyperthermia, like ionizing radiation, arrests cells in G_2/M; however, in hyperthermia, cell arrest occurs earlier and lasts for a during shorter time than does radiation block (Valenzuela et al., 1997).

[1] *Abbreviations*: HSR—homogeneously staining region; AGM—additional genetic material; DM—double minute chromosome; MDR—multidrug resistance; RT—reverse transcription; PCR—polymerase chain reaction.

Table 1. Primer sequences for PCR-assay

Gene	Direct primer	Reverse primer	PCR product size	Annealing temperature,°C	Cycle number
hsc70	5'atccccaagattcagaagct3'	5'ttgatgaggacagtcatgac3'	228	63	22
hsp90	5'aatcggaagaagctttcaga3'	5'gtgcttgtgacaatacagca3'	446	55	23
pgp1	5'tctaaggttgtaggggtttt3'	5'tatcaaaccagctcacatcc3'	220	55	23
β-actin	5'gccgagcgggaaatcgtgcgtg3'	5'cggtggacgatggaggggccg3'	508	70	25

Thus, heat stress, like other stressful factors producing single-strand (Warters et al., 1985) and double-strand (Tomita, 2010) DNA breaks, is a potential inducer of chromosome instability. Detailed cytogenetic assay shows what kind of karyotypic changes arise in cells during long-term growth at a higher temperature.

Cell selection for thermoresistance may be accompanied with the appearance of multidrug resistance (MDR) (Konstantinova et al., 1994). MDR manifestation is coupled with enhanced expression of membrane glycoproteins (Pgps) responsible for cytostatic drug efflux from transformed cells.

The study aimed at monitoring the karyotypic structure and gene expression for heat shock proteins (*hsp70* and *hsp90*) and p-glycoprotein (*pgp1*) in Chinese hamster cells CHL V-79 RJK during long-term cultivation at an elevated temperature (40°C instead of the usual 37°C) to select thermoresistant variants.

MATERIALS AND METHODS

Cells. Lung fibroblasts of Chinese hamster CHL V-79 RJK were kindly provided by Dr. Ruddle (Yale University, United States). The cells were grown in 25-cm^2 flasks (Nunc, Denmak) in DMEM medium (Flow Laboratories, United States) with 10% fetal calf serum (Biolot, Russia) and 100 μg/mL gentamicin (Sigma, United States) at 5% CO_2 at 37°C.

Thermoresistant cells were selected from parental cells proliferating at 40°C. The experimental scheme was as follows. Only a few cells retained viability after 10–12 days in culture at increased temperature. These cells were transferred in fresh medium at 37°C and grown. Cells that reached the stage of logarithmic growth were subcultured (1 : 3) and cultivated at 40°C for 60–80 days. Cells were replated after 48–72 h with 0.25% trypsin (Biolot, Russia). The karyotype of thermoresistant cells was assessed at passages 10, 22, 34, 55, and 75.

Metaphase chromosomes. To accumulate mitotic cells, 3.6 μg/mL colchicine were added to the growth medium. The cells were removed from the substrate with 0.25% trypsin (Biolot, Russia). The enzyme activity was neutralized with conditioned medium, and 0.56% KCl (Reachem, Russia) was used as hypotonic solution (the exposure time varied). The cells were fixed with methanol/glacial acetic acid (Reachem, Russia) mixture (3 : 1). The cooled fixator was changed three times. Total fixation time was 1.5 h. Fixed cells were dropped on wet slides, dried at room temperature, and stained. **Chromosomes were stained** with 2% Giemsa dye (BDH, United Kingdom) in phosphate buffer (pH 6.8). The same dye was used for G-banding, but preparations were preliminarily treated with 0.25% trypsin (Biolot, Russia).

Karyotype was analyzed under an Ampleval light microscope (Germany) at 20× and 100× magnification. Plates with well-spread chromosomes were assayed. The frequency of polyploid cells in the population was found by counting no less than 500 metaphase plates. Chromosomes were identified according to the standard nomenclature proposed for Chinese hamster (Ray and Mohandas, 1975). The frequency of chromosomal rearrangements was determined by counting no less than 20 metaphase plates.

Polymerase chain reaction (PCR) to determine gene expression. Total RNA was isolated according to the routine method (Chomczynski and Sacchi, 1987). One microgram of total RNA was used for cDNA synthesis with the RevertAid H Minus First Strand cDNA Synthesis Kit (Fermentas, Lithuania) in accordance with the manufacturer's instructions. Specific genes were amplified by Taq DNA polymerase (Fermentas, Lithuania) with CycloTemp amplificator (CTM, Russia) and the following program: hot start–denaturation, 93°C, 3 min; primer annealing, 56–70°C, 2 min; elongation, 72°C, 1 min 30 s; than 22–25 cycles: denaturation, 93°C, 45 s; primer annealing, 53–70°C, 1 min; elongation, 72°C, 1 min 30 s; and final elongation, 72°C, 10 min. The PCR conditions and primer sequences are presented in Table 1. β-actin gene was used to control RNA quantity and DNA contamination. The amplified product was subjected to electrophoresis in 2% agarose gel (Sigma, United States) with ethidium bromide (Sigma, United States). A DNA 1 kb kit (Fermentas, Lithuania) was used for molecular mass control. DNA detection was performed with a Transilluminator (UVP, United States) in ultraviolet light (302 nm). Gels were photographed.

RESULTS AND DISCUSSION

Cytogenetic analysis of parental CHL V-79 RJK cells performed during their long-term cultivation

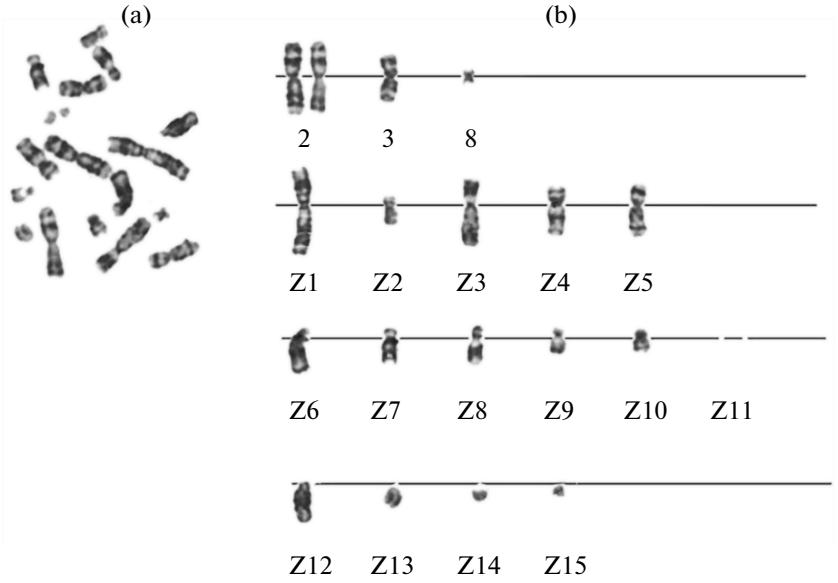

Fig. 1. Standard karyotype of Chinese hamster fibroblasts CHL V-79 RJK. G-banding. (a) Metaphase plate; (b) karyogramm, chromosome number (n) is 18, chromosome 2, 3, 8 do not have abnormalities; Z1–Z15 are markers.

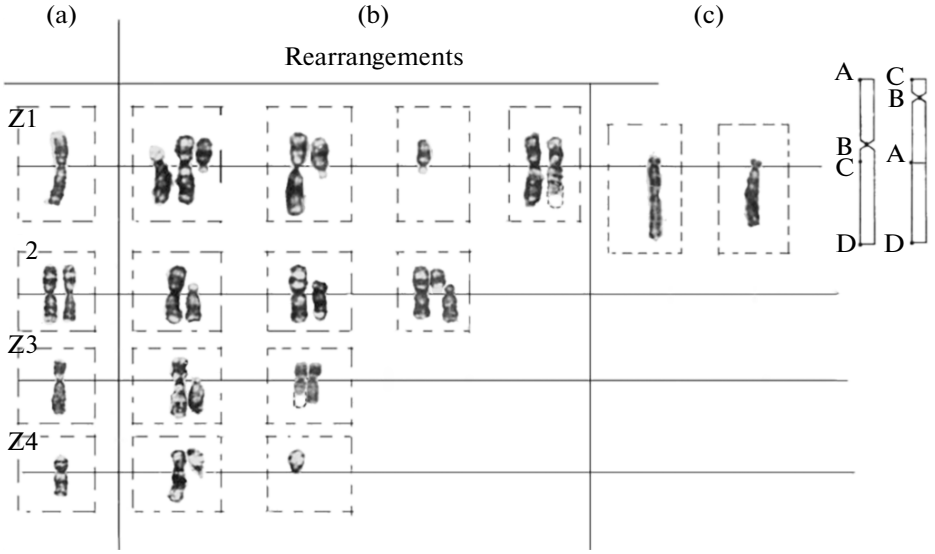

Fig. 2. Random rearrangements in CHL V-79 RJK cells resistant to temperature 40°C at passages 10 and 22. (a) Standard chromosomes; (b) chromosome breaks, passages 10 and 22; (c) inversions, passage 22.

(several years, periodically cells were frozen and thawed) revealed their relatively stable karyotype. Aneuploid cells with chromosome number 18–19 had an advantage. Polyploid near-tri- and near-tetraplod cell variants were of rare occurrence (1–2%). A frequent chromosome set was presented with three unrearranged chromosomes (2, 3, 8) common for normal Chinese hamster cells and 15 rearranged chromosomes (Z1–Z15), which were the cell line markers (Fig. 1). Deviations from this standard were rare and were apparent as the presence of atypical randomly rearranged chromosomes or additional copies of the smallest chromosomes (Table 2).

Short-term CHL V-79 RJK cultivation (10–22 passages) at the temperature increased to 40°C resulted in karyotype instability. At the tenth passage, the frequency of near-tri- and near-tetraploid cells increased to 14% as compared to 1–2% in the parental cell line. Single polyploidy cells had a near-pentaploid chromosome number (within 100). This was never

Table 2. Karyotypic variability of CHL V-79 RJK cells during long-term cultivation at elevated temperature (40°C)

Karyotype structural abnormalities	Cell number with rearrangements						
	total number of analyzed cells						
	37 °C		40 °C				
	50 (10)	50 (75)	26 (10)	34 (22)	28 (34)	26 (55)	23 (75)
Chromosome Z1							
PCB with retained CM (p, q)	–	–	2	–	–	2	–
$Z1^n$ + Z1q	–	–	1	–	1	–	–
$Z1^n$ + Z1p	–	–	–	–	–	–	1
$Z1^n$ + Z1del p ter	–	–	–	–	–	–	1
Break Z1q ter with retained CM	–	–	1	–	–	–	–
del p ter	–	–	–	4	–	–	–
inv pericentric	–	–	–	3	–	–	–
inv paracentric	–	–	–	1	–	–	–
Chromosome 2 (2 copyes)							
$1C^n$; 1C (p, q)	–	–	4	–	1	18	22
$1C^n$ + 1C with pericentric inv	–	–	1	–	–	–	–
2C with PCB (retained CM 2p 2q)	–	1	–	–	1	2	–
$2C^n$ + p	–	–	–	–	–	2	–
$1C^n$ + p	–	–	1	–	1	1	–
$1C^n$ + q	–	–	2	–	–	2	–
$1C^n$ + 2p + q	–	–	–	–	–	1	–
$1C^n$ + 1C del p ter, retained CM	–	–	1	–	–	–	
2^n + q	–	–	2	–	1	–	–
$1C^n$ + 1C with HSR in 2p210–31	–	–	–	–	–	–	22
Chromosome Z3							
PCB with retained CM (p, q)	–	1	–	3	–	1	23
Z3p	–		1	–	–	1	–
Break q ter, retained CM	–	–	1	–	–	–	–
$Z3^n$ + Z3p	–	–	3	1	–	–	–
$Z3^n$ + Z3q	–	–	–	–	–	1	–
ACM (single disk duplication) in near-centromer region qZ3	–	–	–	–	–	–	23
Chromosome Z4							
del q ter	–	–	1	–	–	–	–
$Z4^n$ + Z4p	–	–	3	–	–	–	–
Chromosome 3							
PCB with retained CM (p, q)	–	–	1	–	1	1	–
Chromosome Z5							
$Z5^n$ + Z5p	–		–	–	–	1	–
$Z5^n$ + Z5 with ACM in p ter	–	–	–	–	1	–	–

Passage number is indicated in brackets. HSR—homogenous staining regions, ACM—additional chromosome material; C—copy; PCB—pericentromeric break; CM—chromosomal material; p and q—short and long arms, respectively; ^{n}—normal chromosome; ter—terminal; del—deletion; inv—inversion.

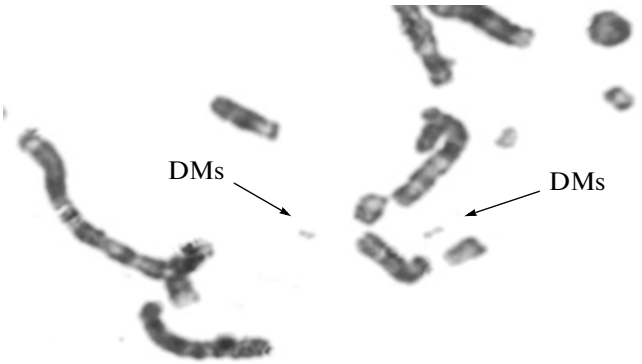

Fig. 3. Metaphase fragment of thermoresistant CHL V-79 RJK cells with double-minute chromosomes (DMs).

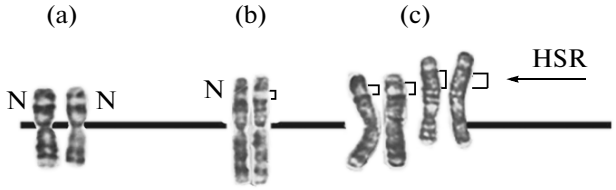

Fig. 4. Structural alterations in chromosome 2 during long-term cultivation of CHL V-79 RJK cells at elevated temperature. (a) Two copies of chromosome 2 in cells under standard culture conditions, homologues have identical structure; (b) modified morphology of 2p210–31 locus in a CHL V-79 RJK cell resistant to 40°C, passage 34; (c) this modified locus in a tetraploid cell, passage 75; brackets indicate an area with modified morphology and HSR. N is normal chromosome morphology.

observed in cells maintained under standard conditions. Detailed analysis of the karyotype structure revealed chromosome breaks (Fig. 2). Chromosomes 2, 3, Z1, Z3, Z4, and Z5 displayed increased fragility (Fig. 2). The most sensitive to hyperthermia was chromosome 2. Breaks occurred frequently in the near-centromere region with p- and q-arms converting individual chromosomes. Some cells had additional third copy 2p or 2q (Table 2). Break points in three other chromosomes during this selection period were irregular. Some cells had DM-chromosomes, which were never observed in the parental cell line.

At passage 22, the frequency of poliploid cells in the population maintained at the increased temperature diminished to the norm (2%). The cell number with changes in the chromosome structure decreased compared to passage 10, but the range of structural modifications increased. Along with the karyotypic instability observed at the tenth passage, cells with novel type rearrangements, para- and pericentric inversions, were registered at passage 22 (Table 2, Fig. 2). These rearrangements were localized in chromosomes 2 and Z1, which exhibited increased fragility at passage 10. The cell frequency with DMs was similar to that registered at the tenth passage. A slight increase in the p10–31 locus length in one of the chromosome 2 homologues was revealed in some cells (Fig. 3a).

At passage 34, rough rearrangements (inversion, deletion) observed at earlier steps of cultivation under hyperthermia (passages 10 and 22), as well as DM-chromosomes, were not registered. The frequency of cells with various deviations was random. However, karyotype destabilization manifested in increased fragility in near-centromere regions of chromosomes 2, 3, Z1, and Z5 (Table 2) was evident. Cells with different lengths of the p210–31 locus in chromosome 2 homologues exhibited an advantage as compared to the previous passage (Fig. 4).

After 55 passages, the cell population was significantly transformed. Most metaphase plates (96%) had near-centromere breaks in one homologue of chromosome 2. 2p and 2q were present as individual chromosomes and became markers (M1 and M2) at this selection stage. AGM in one of chromosome 2 homologues (p210-31) retained unaltered (Fig. 4).

At passage 75, all metaphase plates displayed novel features of destabilization (Fig. 5). Chromosome 2 was presented with only one normal copy. The second copy retained p-arm as a marker chromosome M1. Its length exceeded one-third of the normal 2p due to the increased length of the 2p210–31 locus having a well-defined homogenous staining region (HSR) varied in length. Another feature of this cultivation stage was the presence in most cells of two new marker chromosomes (M3, M4) resulting from a near-centromere break of chromosome Z3 and the appearance of p- and q-arms as individual chromosomes. The pattern of Z3q G-disks was modified by duplication of one of them (Fig. 5). These kariotypic changes were common for all cells.

RT-PCR revealed that thermoresistant CHL V-79 RJK cells at early passages had slightly increased expression of *hsc70*, a constitutive isoform of heat shook protein Hsp70. At passage 38, expression of this protein declined. After 78 passages, a difference was not revealed between thermoresistant and parental cells in *hsc70* expression (Fig. 6). Expression of inducible isoform *hsp90* at the passage 78 did not differ from control cells (Fig. 6b). Expression of *pgp1* gene in thermoresistant cells was higher than at the the control level. Its level of expression changed during further cultivation. At passage 38, it increased, while it returned to the base level at passage 78.

Thus, karyotype of CHL V-79 RJK selected for thermoresistance (40°C) underwent a number of modifications during long-term cultivation. The first stage was an increase in the frequency of polyploid cells and the appearance of randomly rearranged injured chromosomes (with deletions or inversions) and additional genetic material as DM-chromosomes. The second stage was stabilization of the chromosome set caused by elimination of polyploid cells, on one hand; on the other hand, destabilization progressed

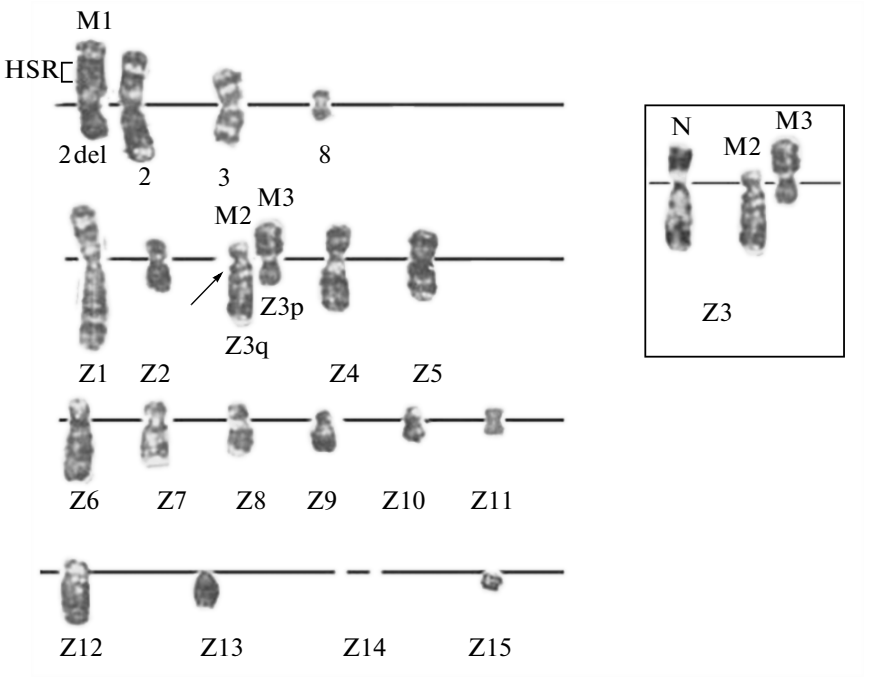

Fig. 5. Metaphase plate of thermoresistant CHL V-79 RJK cell typical for passage 75. M1, M2, and M3 are marker chromosomes arising by near-centromere breaks of chromosomes 2 and Z3. The arrow indicates a duplicated locus. The insert shows generation of M2 and M3 markers.

due to the appearance of novel chromosomal rearrangements (inversions) extending karyotypic variability. The third stage was selection of cells with particular changes and the appearance of specific markers resulting from near-centromere breaks in chromosome 2 and HSR in 2p210–31. The fourth stage was progression of karyotypic instability in cell culture that is resistant to hyperthermia and actively proliferating.

The increased polyploidy (14% versus 1–2% in the norm) and appearance of randomly rearranged chromosomes in CHL V-79 RJK cells at an early passage (passage 10) at elevated temperature is probably a result of heat-shock-produced cell-division disorders. Disturbances in cytokinesis produce rearrangements in the karyotype structure, the appearance of polyploidy cells and morphologically defective chromosomes.

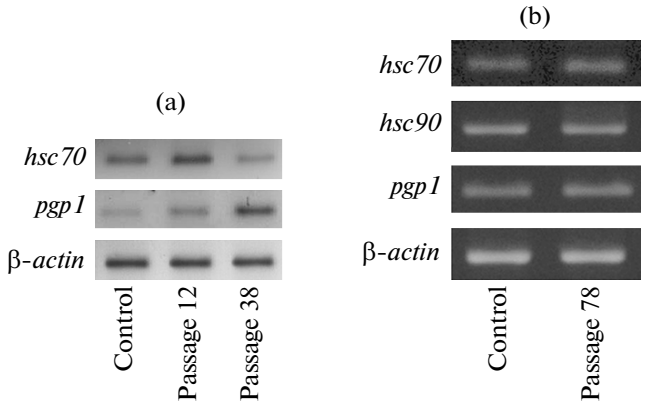

Fig. 6. Expression of (a, b) *hsc70* and (b) *hsp90* and *pgp1* genes at various passages of CHL V-79 RJK cells cultivated at elevated temperature. RT-PCR assay.

It has been proposed that some chromosomes of the karyotypic set are conservative, whereas others have fragile sites (Dekaban, 1965). Our results show that CHL V-79 RJK karyotype also has fragile sites. They are located in large chromosomes mostly in near-centromere regions. Chromosome breaks may determine the fate of the cell population. On one hand, they may promote elimination of centromere-free genetic material in mitosis. On the other hand, they assist in the generation of other aberrations, inversions and translocations. Inversions and translocations change gene sequences that modify their functioning (enhancement or inhibition of their expression) and the genetic status of the original biological material.

It has been proposed that the genetic instability of certain chromosomes under stress conditions suggests the localization of the gene(s) responsible for cell adaptation (transformation) in these chromosomes (Baker et al., 2007). In thermoresistant CHL V-79 RJK cells, chromosome instability (fragility) was attributed to six chromosomes—2, 3, Z1, Z3, Z4, and Z5. Breaks in these chromosomes were registered in the early cultivation period (10–22 passages), but were random. Long-term cultivation under conditions of hyperthermia was accompanied with selective advan-

tage of cells with an abnormal chromosome 2 and, later, Z3. The pattern of these chromosome structural modifications was altered during the cell subculturing. At passage 75, in most cells, the karyotype structure endured gross modifications, with new markers appearing. This suggests that longer cultivation (more 75 passages) instability of chromosomes 3, Z1, Z4, and Z5 may lead to the appearance of new marker chromosomes and their number will increase in time. Manifestation of marker chromosomes in long-term cultivation of CHL V-79 RJK-40 cells under elevated temperature allows us to consider selected thermoresistant cells as a novel cell line.

Heat shock is accompanied with altered Hsp expression. In this study, we assayed gene expression of the HSP70 family in thermoresistant CHL V-79 RJK cells during their long-term cultivation. We found that gene expression of constitutive isoform (*hsc70*) varied at various cultivation steps. RT-PCR assay at an early stage (passage 10) of *hsc70* expression increased, slightly decreased to passage 38, and returned to the background level to passage 78 (Fig. 6). It has been reported that hyperthermia exposure of fibroblasts deficient in inducible Hsp70 isoforms Hsp70 (Hsp70.1 and Hsp70.3) induced chromosome aberrations (Hunt et al., 2004). We showed that karyotypic instability in thermoresistant CHL V-79 RJK was accompanied with increased *hsc70* expression. Our observations, taken together with the literature data, suggest that modified expression of HSP70 family proteins and karyotypic instability are coupled.

Protein Pgp1 is an ATP-dependent membrane transporter engaged in MDR. Its gene contains the regulatory sequence, the heat shock element (HSE), interacting with heat shock factor HSF1. Their binding increased *pgp1* expression. The morphological manifestation of the gene amplifications is HSRs on chromosomes. Cytogenetic assay of CHL V-79 RJK selected on the resistance to ethidium bromide that resulted in MDR showed that the resistant cells underwent karyotypic changes during long-term cultivation (Grinchuk et al., 1988, 1996). The presence of HSRs in chromosomes and breaks in particular loci makes bromide-resistant and thermoresistant CHL V-79 RJK cells similar in the karyotypic changes they underwent during long-term cultivation.

Moreover, CHL V-79 RJK's acquirement of MDR phenotype, as well as thermoresistance, is accompanied with karyotype destabilization involving the same chromosomes 2, 3, Z1, Z3, Z4, Z5. These results suggest that the mechanism of the genome destabilization during cell selection for resistance to elevated temperature and MDR-induced agents is universal.

ACKNOWLEDGMENTS

This work was supported by the Russian Foundation for Basic Research, project no. 14-04-01820.

REFERENCES

Alekseenko, L.L., Zemelko, V.I., Zenin, V.V., Pugovkina, N.A., Kozhukharova, I.V., Kovaleva, Z.V., Grinchuk, T.M., Fridlyanskaya, I.I., and Nikolsky, N.N., Heat shock induces apoptosis in human embryonic stem cells but a premature senescence phenotype in their differentiated progeny, *Cell Cycle*, 2012, vol. 11, pp. 3260–3269.

Baker, D.E., Harrison, N.J., Maltby, E., Smith, K., Moore, H.D., Shaw, P.J., Heath, P.R., Holden, H., and Andrews, P.W., Adaptation to culture of human embryonic stem cells and oncogenesis in vivo, *Nat. Biotechnol.*, 2007, vol. 25, pp. 207–215.

Bhuyan, B.K., Day, K.J., Edgerton, C.E., and Ogunbase, O., Sensitivity of different cell lines and of different phases in the cell cycle to hyperthermia, *Cancer Res.*, 1977, vol. 37, pp. 3780–3784.

Boulon, S., Westman, B.J., Hutten, S., Boisvert, F.M., and Lamond, A.I., The nucleolus under stress, *Mol. Cell.*, 2010, vol. 40, pp. 216–227.

Chomczynski, P. and Sacchi, N., Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction, *Analyt. Biochem.*, 1987, vol. 162, no. 1, pp. 156–159.

Dekaban, A., Persisting clone of cells with an abnormal chromosome in a woman previously irradiated, *J. Nucl. Med.*, 1965, vol. 760, pp. 740–745.

Gasch, A.P., Spellman, P.T., Kao, C.M., Carmel-Harel, O., Eisen, M.B., Storz, G., Botstein, D., and Brown, P.O., Genomic expression programs in the response of yeast cells to environmental changes, *Mol. Biol. Cell*, 2000, vol. 11, pp. 4241–4257.

Grinchuk, T.M., Ignatova, T.N., Sorokina, E.A., Artsybasheva, I.V., and Panshina, Yu.T., Chromosome polymorphism in mammalian cells with multidrug resistance. I. Karyotypic alanalysis of Chinese hamster cells resistant to ethidium bromide at the early passages of initial selection steps, *Tsitologiia*, 1988, vol. 30, no. 3, pp. 312–320.

Grinchuk, T.M., Lipskaya, L.A., Artsybasheva, I.V., Sorokina, E.A., Panshina, Yu.T., and Ignatova, T.N., Karyotype variability in the Chinese hamster lung V-79 RJK cells with multidrug resulting from MDR genes amplification, *Tsitologiia*, 1996, vol. 38, no. 2, pp. 161–172.

Harmon, B.V., Corder, A.M., Collins, R.J., Gobe, G.C., Allen, J., Allan, D.J., and Kerr, J.F., Cell death induced in a murine mastocytoma by 42–47 degrees C heating in vitro: evidence that the form of death changes from apoptosis to necrosis above a critical heat load, *Int. J. Radiat. Biol.*, 1990, vol. 58, pp. 845–858.

Hildebrandt, B., Wust, P., Ahlers, O., Dieing, A., Sreenivasa, G., Kerner, T., Felix, R., and Riess, H., The cellular and molecular basis of hyperthermia, *Crit Rev. Oncol. Hematol.*, 2002, vol. 43, pp. 33–56.

Konstantinova, M.F., Nisman, B.Kh., and Ignatova, T.N., Selection and phenotypic features of heat resistant cells of line CHO-K1, *Tsitologiia*, 1994, vol. 36, no. 2, pp. 182–188.

Kregel, K.C., Heat shock proteins: modifying factors in physiological stress responses and acquired thermotolerance, *J. Appl. Physiol.*, 2002, vol. 92, pp. 2177–2186.

Limoli, C.L., Kaplan, M.I., Phillips, J.W., Adair, G.M., and Morgan, W.F., Differential induction of chromosomal instability by DNA strand-breaking agents, *Cancer Res.*, 1997, vol. 57, pp. 4048–4056.

Morgan, W.F., Day, J.P., Kaplan, M.I., Mc, Ghee, E.M., and Limoli, C.L., Genomic instability induced by ionizing radiation, *Radiat. Res.*, 1996, vol. 146, pp. 247–258.

Palzer, R.J. and Heidelberger, C., Studies on the quantitative biology of hyperthermic killing of HeLa cells, *Cancer Res.*, 1973, vol. 33, pp. 415–421.

Ray, M. and Mohandas, T., Proposed banding nomenclature for the Chinese hamster chromosomes (*Cricetulus gruseus*), *Cytogenet. Cell Genet.,* 1975, vol. 16, pp. 83–91.

Richter, K., Haslbeck, M., and Buchner, J., The heat shock response: life on the verge of death, *Mol. Cell.*, 2010, vol. 40, pp. 253–266.

Tabuchi, Y., Takasaki, I., Wada, S., Zhao, Q.L., Hori, T., Nomura, T., Ohtsuka, K., and Kondo, T., Genes and genetic networks responsive to mild hyperthermia in human lymphoma U937 cells, *Int. J. Hyperth.*, 2008, vol. 24, pp. 613–622.

Toivola, D.M., Strnad, P., Habtezion, A., and Omary, M.B., Intermediate filaments take the heat as stress proteins, *Trends Cell Biol.*, 2010, vol. 20, pp. 79–91.

Tomita, M., Involvement of DNA-PK and ATM in radiation- and heat-induced DNA damage recognition and apoptotic cell death, *Radiat. Res.*, 2010, vol. 51, pp. 493–501.

Van der Waal, R., Malyapa, R.S., Higashikubo, R., and Roti, J.L., A comparison of the modes and kinetics of heat-induced cell killing in HeLa and L5178Y cells, *Radiat. Res.*, 1997, vol. 148, pp. 455–462.

Vogel, J.L., Parsell, D.A., and Lindquist, S., Heat-shock proteins Hsp104 and Hsp70 reactivate mRNA splicing after heat inactivation, *Curr. Biol.*, 1995, vol. 5, pp. 306–317.

Warters, R.L., Brizgys, L.M., and Axtell-Bartlett, J., DNA damage production in CHO cells at elevated temperatures, *J. Cell Physiol.*, 1985, vol. 124, pp. 481–486.

Welch, W.J. and Suhan, J.P., Morphological study of the mammalian stress response: characterization of changes in cytoplasmic organelles, cytoskeleton, and nucleoli, and appearance of intranuclear actin filaments in rat fibroblasts after heat-shock treatment, *J. Cell Biol.*, 1985, vol. 101, pp. 1198–1211.

Welch, W.J., Kang, H.S., Beckmann, R.P., and Mizzen, L.A., Response of mammalian cells to metabolic stress; changes in cell physiology and structure/function of stress proteins, *Curr. Top. Microbiol. Immunol.*, 1991, vol. 167, pp. 31–55.

Westra, A. and Dewey, W.C., Variation in sensitivity to heat shock during the cell-cycle of chinese hamster cells in vitro, *Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med.*, 1971, vol. 19, pp. 467–477.

Wong, R.S., Thompson, L.L., and Dewey, W.C., Recovery from effects of heat on DNA synthesis in Chinese hamster ovary cells, *Radiat. Res.*, 1988, vol. 114, pp. 125–137.

Translated by I. Fridlyanskaya